BRITISH JOURNAL OF CANCER

VOL. I JUNE, 1947 NO. 2

CHANGES IN CARTILAGE PRODUCED BY INFILTRATING CARCINOMA.*

B. SYLVÉN.

From the Department of Radio-Pathology, Radiumhemmet, Stockholm, Sweden.

Received for publication February 11, 1947.

OUR defective knowledge of the biological factors responsible for infiltrative growth is partly due to lack of sufficient information on the biology of the stroma connective tissue in malignant tumours. Thus, growing carcinoma vegetations induce different complicated reactions on the part of the surrounding connective tissue, e.g. fibroblast proliferation and various inflammatory reactions. In the course of the stroma investigations by the writer, special attention has been paid to the transformation ("dedifferentiation") of the connective tissue preceding the actual infiltration by cancer cells and to the simultaneous appearance of high molecular ester sulphates of unknown composition (Sylvén, 1938, 1941, 1945 and unpublished data.) Interpretation of the events taking place in the stroma is rendered almost impossible by the multiplicity of intermingling reactions. On this account it was decided to treat each problem separately. Consequently, I thought it advisable to study these changes in cartilage, a tissue characterized by very slow reparative and reactive capacities. Thus, the actual question was to investigate the morphology and the histochemical changes of normal cartilage when infiltrated by carcinoma cells. In this way comparisons could be made with the stroma reactions of the different tissues, and with the alterations in the chondroitin-sulphate of cartilage and the unknown stroma ester sulphate mentioned above. By selecting suitable cases it was felt that the source of error caused by the connective tissue stroma reaction accompanying cancer vegetations growing from outside the perichondrium, could be avoided.

For the sake of brevity, the readers are referred to current literature regarding the normal morphology of cartilage (Maximow and Bloom, 1938). Some problems concerning the biological role of chondroitin-sulphate in cartilage have recently been reviewed, and more attention has been paid to the physical and chemical qualities of the ester sulphate than is usual by morphologists (Sylvén, in press). Little information regarding the present question is obtained by studying the literature.

The diverging opinions of the old German schools have been reviewed by Ribbert (1911), who himself presented the first detailed and rather dogmatic

* Aided by grants from the Caroline Institute, Stockholm, and Consul General Axel Ax:son Johnson, Stockholm.

description. He stated that cartilage showed a considerable resistance, but after some time it was digested by the growing cancer cells. Ribbert sketched the proceedings in the following way. The cancer cells were believed to induce a proliferation of the perichondral connective tissue accompanied by an inflammatory reaction. The resulting newly formed, growing, connective tissue would then " penetrate " into the ground substance of cartilage, which was " dissolved " and replaced by the tissue mentioned above. After this, the cancer cells were believed to grow into this connective tissue, thereby seemingly infiltrating the cartilaginous tissue proper. Ribbert emphatically denied that cancer cells ever could infiltrate cartilage directly, the process instead being mediated by connective tissue. As to the operating forces Ribbert stated that " . . . *die Epithelien* (cancerous) *haben nicht die Fähigkeit anders als durch den Wachstumsdruck auf ihn* (cartilage) *einzuwirken.*" Such a restricted mechanical interpretation involving pressure for the explanation of infiltrative growth, is nowadays of little value (Sylvén, 1945).

Oestreich (1910) suggested that the chemical composition of cartilage, mainly the chondroitin-sulphate, would be injurious to the cancer cells and consequently infiltrative growth would become impeded by this substance.

Since Ribbert's review in 1911 no special articles have been published regarding these questions. The statements of Ribbert demand a survey of the cooperating factors active in the destruction of cartilage by carcinomatous tumours. The following possible factors have to be considered :

1. Alterations of the perichondral connective tissue might influence the nutrition of the corresponding cartilage area, thereby causing degenerative changes.

2. The different types of " stroma reaction " (such as young and growing fibroblasts and granulation tissue containing inflammatory cells) might by different means have a deleterious effect on cartilage. These conditions have recently been reviewed with special reference to the inflammatory destruction of cartilage (Sylvén, in press).

3. The cancer cells might by direct action be destructive.

In order to obtain a satisfactory understanding of the morphological factors they should be interpreted in terms of biochemical principles. Unfortunately for lack of complete data the chemical principles can only be touched upon.

Cartilage has a strongly acid intercellular matrix, due to the presence of large amounts of chondroitin-sulphuric acid. This substance is very sensitive to even slight *changes in pH towards the alkaline side* (Blix and Snellman, 1945). Such pH variations cause a rapid depolymerization and disintegration of the long chain molecule. A shift in pH resulting in a disappearance of chondroitin-sulphate has recently been suggested to occur in inflammatory lesions of cartilage (Sylvén, in press). The same mechanism may also operate in carcinomatous destruction of cartilage, evoked either by the stroma reaction or by the cancer cells.

In a general way, we are forced to suppose that the actual destruction, viz. digestion, of cartilage matrix and cells must be an enzymatic process. *Proteolytic enzymes* may effect such tissue changes. These enzymes may derive either from the cells of the stroma reaction or from growing cancer cells. This suggestion has recently gained some support by studies on the infiltrative growth of mesenchymal tumours (Sylvén, 1945).

Thus, among the possible co-operating factors responsible for cartilage destruction, we have to emphasize particularly the changes in hydrogen ion concentration and the appearance of proteolytic enzymes.

MATERIAL AND METHODS.

Some surgically treated cases of carcinoma of the external ear were selected. A selection had to be made in order to avoid very advanced and ulcerated cases with pronounced suppuration. No preoperative irradiation therapy was given. Fresh resected material was fixed in 8 per cent formaldehyde solution for 24–48 hours. Sections were stained in 0·1 per cent toluidine blue in alcohol of varying concentrations (1, 30, 50, 60 and 80 per cent), and further, the common connective tissue staining methods were used. For technical details the reader is referred to earlier publications (Sylvén, 1945).

RESULTS.

A short account of the original findings is presented in Table I. When considering the complexity of the problem, I have to admit that no final conclusions regarding the biochemical nature of the operating factors can be drawn from the material presented here. Some results, however, seem to be conclusive.

Some information concerning the mutual relationship between cancer cells and the perichondral connective tissue in the absence of any intervening inflammatory stroma reaction was obtained from Case 2, II. The carcinoma strands in question were growing in the perichondrium, which was transformed into a loose metachromatic stroma connective tissue similar to the one met with in most carcinomas (Sylvén, 1938, 1945 and unpublished data.) In the superficial part of the underlying cartilage a moderate decrease in the content of chondroitin-sulphate was demonstrated, the cartilage in other respects apparently being normal. No infiltration of the cartilaginous tissue proper was seen in a considerable area. The observed decrease in intensity of the metachromatic staining reaction was strictly limited to that part of cartilage underlying the cancerous infiltration of the perichondrium.

In two cases of squamous-cell carcinoma (No. 4 and 5) growing cancer vegetations without any accompanying inflammatory stroma reaction were seen to be mingled with cartilage cells in the process of being digested (Fig. 6–9). In this area of cartilage the chondroitin-sulphate of the intercellular medium was lacking, apart from some small pericellular halos of metachromatic material here and there (Fig. 4–6). All transitions were seen from normal cartilage cells to chondrocytes in necrosis and liquefaction.

In the remaining cases the destruction of cartilage was apparently brought about by the combined activities of the inflammatory stroma reaction and the cancer vegetations. The cartilage changes were gradual, increasing with the advance of the tumorous process, and thus may be described in stages. From Table I (Case 3) it can be seen that the inflammatory stroma reaction which precedes the growing carcinomatous buds, by penetrating the perichondral connective tissue, has the ability to reduce the content of chondroitin-sulphate in the superficial area of cartilage which is otherwise unchanged (Fig. 1). Apparently as an early sign of the advancing tumour the content of chondroitin-sulphate may be diminished by the action of lymphocytes and plasmocytes (Case 4 and 5).

Actual cartilage destruction was seen to be effected either by the sole inflammatory stroma reaction (Case 1) or by the combined stroma and cancer strands (Case 2). In the cases under consideration, that part of cartilage just being infiltrated was always devoid of the typical ester sulphate. Remains of this substance might be found in the shape of thin pericellular halos. When moving from the area of destruction towards the neighbouring unchanged cartilage a gradual increase in the content of chondroitin-sulphate was observed (Fig. 1, 3–6). It may be mentioned that the destructive process seemed to be more rapid and violent in cases of squamous-cell carcinoma than in the basal-cell carcinomas.

DISCUSSION.

In a previous paper a localized chronic perichondritis was found to cause a decrease in the content of chondroitin-sulphate in the underlying part of ear cartilage. A similar condition is apparently evoked by a carcinomatous transformation of the perichondral connective tissue, as stated above. It may be suggested that this alteration of the perichondrium affects its normal function, whereby nutrition of the corresponding cartilage becomes impaired. This alteration of cartilage, previous to the actual infiltration by cancer cells and previous to established inflammatory changes was not observed before.

EXPLANATION OF PLATES.

FIG. 1.—The early changes in ear cartilage, previous to the actual infiltration, are demonstrated. At top of picture the perichondral connective tissue is swollen and slightly invaded by lympho-plasmocytes. The superficial part of cartilage appears unstained, indicating that metachromatic material is lacking. At bottom of picture normal cartilage, rich in metachromatic substance, stained in dark black colour (violet in the slide). Case 4. (Toluidine blue, × 90.)

FIG. 2.—The destruction of cartilage is now going on by the combined action of the inflammatory stroma reaction and the cancer vegetations. To the right intact cartilage is seen. The ester sulphate content cannot be estimated. Case 4. (Connective tissue stain of Hansen, × 50.)

FIG. 3.—The same picture as Fig. 2. The metachromatic staining reaction reveals the distribution of the chondroitin-sulphate. Note the total depletion of metachromatic substance in the left part of cartilage just invaded. Case 4. (Toluidine blue, × 50.)

FIG. 4.—The disappearance of chondroitin-sulphate precedes the growing tumour. In this picture the stroma reaction has reached the left top corner; the lightly stained ametachromatic cartilage area contains very few inflammatory cells. At bottom of the picture disintegration of ester sulphate is proceeding. Case 4. (Toluidine blue, × 90.)

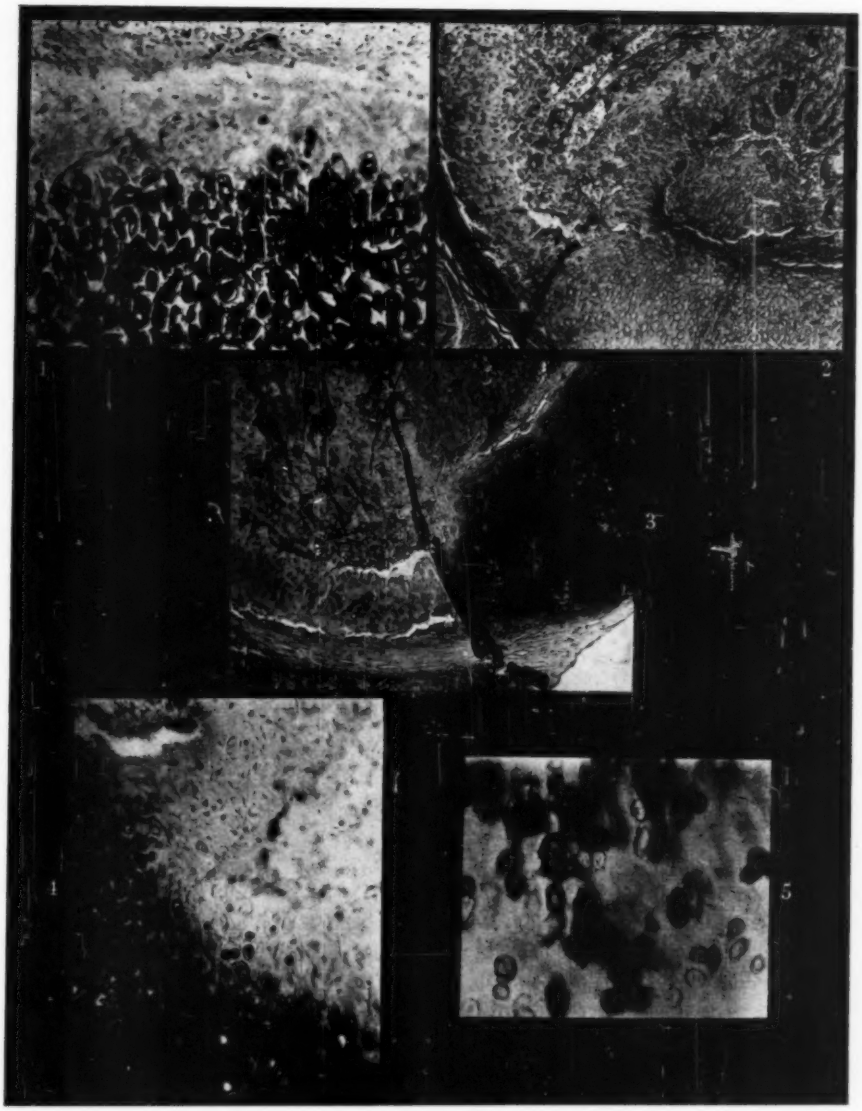
FIG. 5.—From the same zone of cartilage as in Fig. 4. The largest part of chondroitin-sulphate has disappeared from the cartilaginous matrix, but still some chondrocytes are surrounded by metachromatic halos, which appear black (violet in the slide). Case 4. (Toluidine blue 0·1 per cent in alcohol 70 per cent, × 210.)

FIG. 6.—Vegetations of squamous-cell carcinoma devoid of any demonstrable connective tissue stroma, actively infiltrating cartilage, which is depleted of chondroitin-sulphate. Case 4. (Toluidine blue, × 90.)

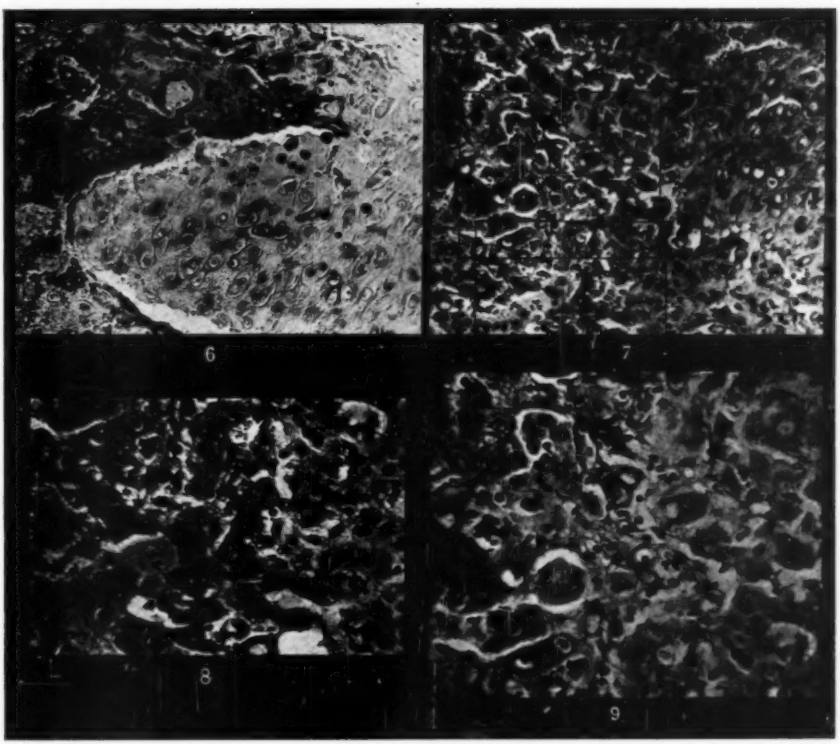
FIG. 7.—Growing cancer vegetations mingled with fragments of cartilage just being digested. A small number of lympho-plasmocytes is present. No connective tissue stroma reaction could be demonstrated. Case 4. (Connective tissue stain of Hansen, × 120.)

FIG. 8.—Greater magnification of area marked in Fig. 7. Remnants of chondrin balls are in contact with growing cancer cells. Case 4. (Connective tissue stain of Hansen, × 210.)

FIG. 9.—Another area marked in Fig. 7. The absence of connective tissue stroma and scattered lympho-plasmocytes are noted. This picture demonstrates the morphology of scattered cancer vegetations breaking up a compact block of cartilage (devoid of ester sulphates). Case 4. (Connective tissue stain of Hansen, × 210.)



Sylven.



Sylven.

TABLE I.—*Changes in Cartilage Produced by the Action of Infiltrating Carcinoma.*

Case No.	Type of carcinoma.	Age of patient.	Type of stroma reaction.	Content of chondroitin-sulphate.
1	Basal-cell	65	Intense lympho-plasmocytic infiltrations extending into perichondrium and cartilage. Tumour vegetations have not yet reached the cartilage.	Beneath the inflammatory deranged perichondrium a moderate decrease in chondroitin-sulphate is observed. Where cartilage destruction is seen effected by the stroma reaction all ester sulphate has disappeared.
2	„	65	I. In a small area the combined inflammatary stroma reaction and cancerous vegetations are eroding cartilage.	I. In this place cartilage is devoid of chondroitin-sulphate.
			II. In an area 2 mm. × 0·1 mm. cancer cells spread in the perichondral connective tissue without affecting the underlying cartilage. No inflammatory cell reaction present. Transformation of perichondral tissue, but no fibroblast proliferation.	II. The underlying cartilage presents a moderate decrease in chondroitin-sulphate content to a depth of about 1·0 mm. Other changes in cartilage texture are not demonstrable.
3	„	65	Marked perichondral infiltration by lympho-plasmocytes. Cancer cells have not yet reached the cartilage.	In the superficial part of the cartilage area, a moderate decrease in the content of chondroitin-sulphate is seen. Chondrocytes somewhat swollen, matrix otherwise unchanged.
4	„	78	The deep tumour vegetations are accompanied by a moderate lympho-plasmocytic reaction, which is covering a large area of cartilage. Destruction of cartilage only in one circumscribed central area, where the inflammatory stroma reaction is very insignificant, and cancer nests are mingled with cartilaginous tissue.	The inflammatory stroma reaction has induced a moderate decrease in the content of chondroitin-sulphate. In area of cartilage destruction practically all chondroitin-sulphate is lost. Cancer cells are directly in contact with bars of partly eroded cartilage (Fig. 1–9).
5	„	79	Heavy infiltration by lympho- and leucocytes. Extensive erosion of cartilage with a large perforation.	The cartilage bordering the tumour is reduced in thickness and contains less chondroitin-sulphate, which still appears in isolated places as pericellular halos. In areas of erosion actual contact between cartilage and cancer cells is seen, and no chondroitin-sulphate is present in the areas where the chondrocytes are being digested.

The actual destruction of cartilage generally seems to be effected by the conjoined action of the inflammatory stroma reaction and the cancer cells. So far, Ribbert's statements of 1911 seem to be correct. When investigating the process more carefully, however, it was established *that cancer cells really have the capacity of digesting cartilage by their own activities* without any co-operating stroma reaction. Further, the results indicate, that the first demonstrable alteration of cartilage just being infiltrated is an early disappearance of most of the pre-existing ester sulphate. A moderate infiltration by leucocytes or lympho-plasmocytes is accompanied by a gradual depletion of this substance.

In all probability the observed decrease in the content of chondroitin-sulphate does signify the disintegration of this substance in the same manner as is effected in physiological ossification (Sylvén, in press) and in inflammatory cartilage destruction (Sylvén, in press). As mentioned above, the disintegration might easily be caused by the alkaline reaction, which is known to prevail both in inflammatory media and in the intercellular medium of cancerous vegetations. Judging from the actual destruction and digestion of cartilage, the action of proteolytic enzymes has to be postulated. Apparently, such enzymes may be produced by the cell elements of the preceding stroma reaction (fibroblasts and inflammatory cells), as well as by the growing cancer cells. Obviously the simple mechanical interpretation (Ribbert, 1911) of the factors involved in infiltrative growth processes is incorrect. The present results call for additional data as to the mechanism under question.

Returning to the introduction, some further aspects have to be mentioned. From a series of histochemical investigations on the occurrence of high molecular ester sulphuric acids (or more correctly, " ester sulphates ") in connective tissue during different biological conditions it was found that ester sulphates of unknown composition were demonstrable in the growing tissue of healing wounds, in most mesenchymal tumours, and in the stroma connective tissue of growing epithelia, both normal and neoplastic (Sylvén, 1938, 1941, 1945). Certain observations favoured the opinion that these ester sulphates were derived from the granules of the mast cells, and consequently their composition was suggested to be related to the mucoitin-polysuphuric acid of the mast cells granules. But according to the opinion expressed by Meyer and Chaffee (1941) and Blix and Snellman (1945) the ester sulphates in question were possibly composed of chondroitin-sulphate. Their suggestion was not supported by any experimental evidence, but was only advanced as an analogy between the ordinary connective tissue and cartilage, which are both derived from the embryonal mesenchyme. However, by using the present results the matter in dispute may be further elucidated.

On considering the present findings it seems obvious that cancer cells growing in cartilage are not met with a metachromatic ester sulphate reaction such as is met with when growing in connective tissue. On the contrary, *the preformed ester sulphate*, viz. *chondroitin-sulphate, is rapidly destroyed* either very early by the stroma reaction or directly by the growing cancer vegetations. This difference lends some support to the assumption that *the chondroitin-sulphate is never in contact with the growing cancer cells*. This substance, instead, undergoes disintegration, which shows its stability under the conditions prevailing in the intercellular medium of growing cancer vegetations (hydrogen ion concentration or enzyme content). Further, paying consideration to the extreme susceptibility of the chondroitin-sulphate molecule to even weak alkaline reactions (Blix and Snellman, 1945), it seems improbable that the molecule even could exist in the ordinary intercellular medium of the connective tissue (Sylvén, in press). Thus, the present investigation supports the suggestion that the ester sulphates typical of the stroma of growing carcinoma vegetations have a composition different from that of chondroitin-sulphate.

It may be added, that as yet cancer cells have not been cultivated in media rich in chondroitin-sulphate. Media containing mucoitin-polysulphuric acid (heparin) were introduced by Fischer and Paker.

CONCLUSIONS.

A reinvestigation of the morphology and possible mechanisms of infiltrative-destructive growth by carcinoma into cartilage is presented. The early disintegration of the pre-existing chondroitin-sulphate is emphasized. The active principles are discussed from the morphological and biochemical points of view. A comparison between the reactions of cartilage and the corresponding stroma reaction of the loose connective tissue favours the suggestion that the unknown ester sulphates of the last-mentioned tissue are of another composition than chondroitin-sulphate.

REFERENCES.

BLIX, G., AND SNELLMAN, O.—(1945) *Ark. Kemi Min. Geol.*, **19A**, No. 32, 1.
MAXIMOW, A. A., AND BLOOM, W.—(1938) 'A Textbook of Histology.' Ed. 3. Philadelphia and London (Saunders).
MEYER, K., AND CHAFFEE, E.—(1941) *J. biol. Chem.*, **138**, 491.
OESTREICH, R.—(1910) *Berl. klin. Wschr.*, **47**, 1698.
RIBBERT, H.—(1911) 'Das Karzinom des Menschen, sein Bau, sein Wachstum, seine Entstehung.' Ed. 1. Bonn (Friedrich Cohen), p. 204.
SYLVÉN, B.—(1938) *Klin. Wschr.*, **17**, 1545.—(1941) *Acta chir. scand., Suppl.* 66, p. 1.—(1945) *Acta radiol., Stockh., Suppl.* lix.—(1947) *J. Bone Jt. Surg.* (in press).

REGIONAL INFLUENCES IN CANCER.

D. B. CRUICKSHANK.

From the Sims Woodhead Memorial Laboratory, Papworth Village Settlement, Cambridge.

Received for publication February 15, 1947.

I. METHOD OF ANALYSIS.

A METHOD of analysing the "pattern distribution" on graded county maps whereby the existence, intensity and extent of regional influences could be measured (Appendix 1) has already been described by Cruickshank (1940). The present paper examines the information obtained by the application of the method.

Maps analysed are :—

Maps 1–17 : British Empire Cancer Campaign : *Annual Report*, 1936.
„ 18–23 : „ „ „ „ „ „ 1937.
„ 25–33 : „ „ „ „ „ „ 1939.

These show the geographical distribution of the relative mortality, fully corrected for age and class of district, from cancer of various organs. With two exceptions (Map 14, without County Boroughs ; Map 15, Rural Districts only), the "County" includes the County Boroughs.

The reference numbers used in the Tables correspond to those in the original publications ; the short titles are a guide to classification, but for serious study

TABLE I.—*Gives the Probability of Obtaining by Chance a Degree of "Pattern" Development Equal to or Higher than that Actually Observed on the Maps.*

Number.	Maps. Short description.			Grade (1 = White; 7 = Black).							Intergrade (combining grades).					
				1.	2.	3.	4.	5.	6.	7.	1,2.	2,3.	3,4.	4,5.	5,6.	6,7.
1	Oesophagus.	M	25–65	·66	·71	·27	·70	1·00	·34	1·00	(·06)	·23	·29	·51	(·01)	(·07)
2	Stomach.	M	25 up	*1·00*	·03	(·02)	(·24)	·13	1·00	(·01)	·03	·07	(·11)	·22	·02	< ·01
3	,,	F	25 up	1·00	·11	(·35)	·18	(·04)	·10	< ·01	(·11)	(·11)	·03	(·10)	·04	< ·01
4	,,	P	25–65	*1·00*	·01	·02	·52	(·07)	1·00	< ·01	·01	(·04)	(·11)	·10	·02	(·01)
5	Intestines.	F	25–65	*1·00*	·52	(·03)	·68	·34	1·00	*1·00*	·52	·11	*c.* ·29	·70	·07	1·00
6	,,	F	65 up	·08	·05	(·53)	·04	·29	1·00	*1·00*	(< ·01)	(·03)	*c.* ·29	·60	·04	1·00
7	Rectum.	M	25–65	·38	·52	·22	·03	·82	1·00	·19	(·37)	·19	(·64)	·70	·35	·18
8	,,	M	65 up	1·00	(·16)	(·07)	·05	< ·01	·05	·08	(·04)	·02	·55	(·01)	< ·01	(< ·01)
9	Liver, etc.	F	25–65	·08	(·12)	(·19)	·03	·06	*1·00*	*1·00*	·10	·59	(*c.* ·07)	< ·01	·06	*1·00*
10	Skin.	M	25 up	1·00	(·07)	(·06)	·35	(·22)	1·00	1·00	< ·01	·26	·35	·60	(·64)	1·00
11	Lung.	M	25 up	(·14)	·89	·79	1·00	·19	1·00	(·01)	·80	(·26)	(·93)	(·59)	·18	·03
12	Breast.	F	25–65	*1·00*	·04	(·17)	(·02)	·38	*1·00*	*1·00*	·04	·45	*c.* ·29	(·09)	(·07)	*1·00*
13	,,	F	65 up	·08	·05	·74	·03	(·07)	*1·00*	*1·00*	·06	(·15)	(*c.* ·20)	·03	(·18)	*1·00*
14	Oesophagus.*	M	25 up	·34	·15	·15	(·01)	< ·01	·06	*1·00*	·04	·05	·05	< ·01	< ·01	(< ·01)
15	Various.† (3, 5, 9) above.	F	25 up	*1·00*	·38	(< ·01)	(·09)	(·01)	1·00	·01	·38	(·04)	(*c.* ·20)	·05	·13	(< ·01)
16	All sites.	M	25 up	1·00	·22	·25	·74	(·15)	·02	·38	·21	·19	·45	·01	·06	·02
17	,,	F	25 up	1·00	(·31)	·25	·44	(·01)	·08	1·00	·15	·09	(·29)	(·31)	·07	·05
18	Tongue.	M	25 up	< ·01	·04	·23	·22	(·59)	*1·00*	*1·00*	·05	(·01)	·27	·92	·67	*1·00*
19	Tonsil and mouth.	M	25 up	·13	·92	(·41)	·66	·29	1·00	*1·00*	·46	(·27)	·55	·11	(·21)	1·00
20	Jaw.	M	25 up	·08	·13	·10	(·28)	·34	·38	·38	·07	·08	(·30)	·88	·27	·34
21	Larynx.	M	25 up	·13	(·31)	·13	< ·01	< ·01	1·00	*1·00*	·55	(·38)	·04	< ·01	(·01)	*1·00*
22	Bladder.	M	25 up	·18	·47	·02	·47	·06	·13	1·00	·27	·04	·07	·13	(·02)	·02
23	Prostate.	M	25 up	(·01)	(·59)	·03	·47	1·00	·05	1·00	(·21)	(·25)	(·27)	·46	(·02)	(·04)
25	Uterus.	F	25–65	·08	·34	< ·01	·94	·82	·34	*1·00*	·22	(·03)	(·19)	·94	·13	·34
26	,,	F	65 up	(< ·01)	1·00	(·12)	·09	·03	·01	*1·00*	(·01)	(·26)	(·33)	·19	< ·01	·01
27	Vagina and vulva.	F	25 up	(·07)	·67	·51	·83	·02	·58	< ·01	(·14)	·89	·60	·63	·02	(·02)
28	Ovaries and F.T.	F	25 up	·34	·07	(·24)	·13	·47	1·00	1·00	·04	(·17)	(·16)	·19	(·19)	·52
29	Skin.	F	25 up	·05	(·48)	·67	·13	·67	1·00	(·01)	·13	(·05)	(·04)	(·15)	·77	(·01)
30	Lung.	F	25 up	·10	1·00	(·31)	·22	1·00	·82	·01	·71	(·17)	(·06)	·03	·27	(·31)
31	Rectum.	F	25 up	1·00	·19	·19	·01	(·22)	*1·00*	*1·00*	·06	·19	*c.* ·33	·05	(·16)	*1·00*
32	All sites.	F	25–65	1·00	·82	< ·01	·12	·52	·58	·19	·07	·01	·17	(·01)	·80	·03
33	,,	F	65 up	·01	·38	(·24)	·60	·11	·38	·05	·01	(·13)	*c.* ·20	(·05)	·07	·03

* = without County Boroughs.
† = Rural districts only.

·66 etc. = P_{ng}.
(·02) etc. = P_{nn1}.

1·00 = Single County.
c = Extrapolated Png.

Dr. Stocks' papers should be consulted. All the original maps are by Dr. Percy Stocks.

II. CALCULATING THE DATA.

The pattern of every grade (and intergrade) on these maps is analysed and recorded separately (Appendix 2 and 3).

From these data the procedure is firstly to determine the probability of the *grade pattern as a whole* (P_{ng} of Appendix 1) and, secondly, to determine that of the *largest group* in the grade (P_{ng1} of Appendix 1). The first method is by far the more sensitive (i.e. P_{ng} usually $< P_{ng1}$), and this is the value generally given in Table I.

There are, however, instances, usually when n counties of a grade form one large group, n_1, of *nearly* n counties, in which the second method becomes more sensitive (i.e. $P_{ng1} < P_{ng}$). Where this is the case this value is entered in Table I but shown *within brackets*.

Thirdly, with the object of studying the *interrelationship of the patterns of consecutive grades*, consecutive grades are combined to form "intergrades." Each intergrade is then treated exactly as if it were one large grade and analysed according to the methods given above.

III. PERCENTAGE OF SIGNIFICANT PATTERNS.

The entries in Table I are calculated by the above methods, and give the best available estimate of the probability of obtaining by chance a degree of patterning (i.e. a degree of contiguity of similarly graded counties) equal to, or greater than, that actually observed on the map itself.

The grade and intergrade values are listed separately in the Table, but should be read alternately, viz. 1, 12, 2, 23, 3, 34 . . . 67, 7. The method of analysis employed compensates fully for difference in "size" of the pattern analysed, and the P values for grade and intergrade patterns can therefore be regarded as strictly comparable.

If we regard $P < \cdot 05$ as evidence of abnormal patterning, then a glance at the Table shows that abnormal patterns occur with considerable frequency. This is best seen by comparing the frequency distribution of the 224 grade and 192 intergrade P values of the Table with the strictly equivalent frequency distribution of the 4000 random series (Cruickshank, 1940). We find—

TABLE II.—*Percentage of Significant Patterns* ($P < 0{\cdot}05$).

4000	Random series . . .	3·05 per cent.
224	Grades	20·5 ,,
192	Intergrades	33·4 ,,

Thus one fifth of the grade- and one third of the intergrade-patterns are *significant*.

The increased frequency is not limited to "significant" patterns only, but extends also to patterns of intermediate probability (·25–·05), the frequency of these being much higher in the "map" than in the "random" series (Table III). In fact, the whole disposition of the P values of the "map" and "random" series show marked and systematic divergence (Fig. 1).

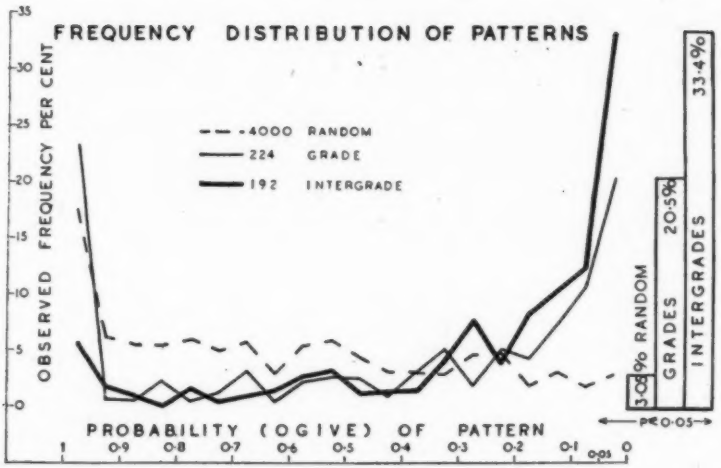


FIG. 1.

Statistical maps often present data by the use of "grades." The several counties in any one grade may be scattered "at random" over the map, or may be juxtaposed to form small or large "groups." These varying types of distribution are described briefly as "patterns." The exact type of pattern to be expected from random distributions has been determined experimentally (4000 patterns), and from these experiments the "expected frequency" of any specific type of observed pattern is known. Rare types of patterns, i.e. patterns with low expected frequency, are described as "highly developed."

On cancer maps highly developed patterns occur with excessive frequency. Indeed, "statistically significant" patterns, i.e. patterns with an expected frequency of $P \leq \cdot 05$, form between 20·5 and 33·4 per cent of all the 416 patterns analysed (224 and 192). Such patterns must be developed under the influence of some non-random factor, and as its effect is to introduce a tendency towards geographical juxtaposition (or grouping) of counties of similar or allied grading, the factor is described as a "Regional Influence."

TABLE III.—*Pattern Frequencies per cent.*

	$P =$ 0·25.	0·2.	0·15.	0·1.	0·5.	·01.
4000 Random series	4·8	2·0	3·3	2·1	3·05	
224 Grades	5·3	4·5	7·5	10·7	20·5	
192 Intergrades	4·2	10·8	10·5	12·5	33·4	

IV. THE MEAN VALUES OF PATTERN PROBABILITY.

Another figure of interest is the arithmetic mean of the P values in Table I. A simple average would suffice, but actually a weighting proportional to the number of counties in the grade (or intergrade) was used. The results are summarized in Table IV.

TABLE IV.

Pattern analysed.	Number analysed.	Mean P value.	S.D.	S.E.
Random	4000	·6165	·2729	·00431
Grade (maps 1–33)	210	·384	·345	·0238
Intergrade (maps 1–33)	192	·249	·269	·0194

These mean P values may appear at first sight rather high, but it must be remembered that—

(*a*) *All* the P values in Table I err on the side of under-statement; the method used expresses the P value of the *general* characteristics of the patterns and quite overlooks more detailed abnormalities.

(*b*) This understatement is even more marked in the case of P values less than ·01; e.g. the direct computation of the P values for the large group in maps Nos. 3 and 4, using special mathematical formulae, shows these to be of the order $P = ·000{,}002$; yet, these are *recorded* merely as $P = <·01$.

Even with these understatements the evidence of non-randomness is overwhelming; the comparisons in Table V show that the mean P values for the grade and intergrade differ from the random value by respectively 9·61 and 18·5 times the S.E. difference.

TABLE V.

Comparison.	Difference.	S.E.$\triangle$.	$\triangle$/S.E.
Random with grade	·233	·02419	9·61
Random with intergrade	·368	·01989	18·5
Grade with intergrade	·135	·03072	4·4

Notice, too, that the patterning in the intergrades is significantly more developed than that in the grades. This should be compared with the higher proportion of significantly patterned areas in intergrades already noted (Section III). As the method of pattern assessment used for grade and intergrade are strictly comparable, these differences indicate a real tendency towards increasing pattern development with increasing area (Appendix 4).

V. THE TREND OF P VALUES ACROSS THE GRADES.

Fig. 2 and 4 are constructed from the data of Table I.

The levels of significance marked on the curves are as follows:

Above upper datum	$P = <·01$
At upper datum	$P = ·01$
At lower datum	$P = ·05$.
Base line	$P = 1·0$.

There are $7 + 6 = 13$ "points" on each curve.

Preliminary drawings indicated that intergrade–intergrade "trends" (i.e. 12–23–34 . . . 67) are generally more regular than grade–grade (1–2–3–4 . . . 7) or "mixed" (1–12–2–23–3 . . . 67–7) trends. This is because intergrade values represent a two-point moving-average across the grades, and are also larger than grades and therefore a sampling unit less subject to random variations.

Intergrade trends are therefore particularly useful as a criterion of random (i.e. by virtue of the method employed), as opposed to true variations in grade P values, and the P curves as presented are smoothed to that criterion, so bringing the essential features of the curves into relief and facilitating comparison with the "zones" on the maps.

A study of Fig. 2 and 4 will show that P values across the gradings (1–12–1–23 . . . 7) give rise to curves with clearly defined trends, which include the develop-

ment of well-marked maxima and minima. The curve type can be classified in terms of the number and position of the maxima.

TYPE 1. Simple form. High at both extremes. Smooth transitions. Maps 2.4.26.27.33.

TYPE 2. As above, but peaks develop before the extremes are reached and then fall off at one or both ends. 1.5.8.22.23.

TYPE 3. Simple form. High at means. 9.13.21.

TYPE 4. Complicated form, three well developed peaks, two at extremeness and one at mean (possibly combines Types 1, 2 and 3). 6.15.25. 30.31.32.

TYPE 5. Curve declines from one extreme to the other. All show minor intercurrent or terminal peaks. 3.10.11.18.28.

TYPE 6. Miscellaneous modifications of above types.

The most striking general feature is the relative simplicity of the curves, for in 17 cases these consist simply of two main peaks; in the remaining 15 cases there is but one additional minor or major peak.

The majority of curves have peaks or plateaux at or near one or both extremes (see Appendix 8). This indicates a positive correlation between increasing deviation of relative mortality and the degree of pattern formation.

This type of correlation is not obligatory, for many curves show that the regional influence modifies the rate to some intermediate value, in which circumstances there will be a negative correlation between pattern formation and increasing deviation.

VI. TOPOGRAPHICAL DISTRIBUTION OF THE SIGNIFICANTLY PATTERNED AREAS.

Fig. 3 and 5 show the topographical distribution of the counties in the "significant" grades (or intergrades) of Table I and Fig. 2 and 4, including, in addition, the topographical distribution of all counties whose relative mortalities deviate in excess of 2 S.E. (see Appendix 5).

The system of shading employed is explained in Section VII.

While these distributions range from (*a*) scattered isolated counties through (*b*) small and (*c*) large areas of significance to cases where (*d*) the whole 62 counties participate in abnormal pattern formation, the main feature is the frequent occurrence of *large zones* of significant patterning.

The position is summarized in Table VI, which shows that all but three of the maps have one or more large zones of significance. The average size of a major zone is 26 counties (i.e. 2/5ths of map) and of a minor zone 13 counties (i.e. 1/5th

TABLE VI.—*Features of Significant Zones.*

Number of zones.	Number of maps with.	Mean size number of counties.	Mean size of separate zones. Major.	Minor.			Broad summary area of significance.
0	3	0	(0)	..	..	..	
1	13	23	(1)	23	..	..	2/5th of map
2	14	41	(2)	28	13	..	3/5ths or more of map
3	2	54	(3)	31	16	7	3/5ths or more of map
All	32	31	26(±8)	13(±8)	7		1/2 of map

(*N.B.*—Additional data from 2 S.E. deviations in relative mortality *not* here included.)

of map). Generally speaking *2/5ths or more* of the area of the map participate in *significant* pattern formation.

The consistent presence of these larger zones seems to indicate that the *distribution* of Regional Influences evolves around some fairly simple theme. If

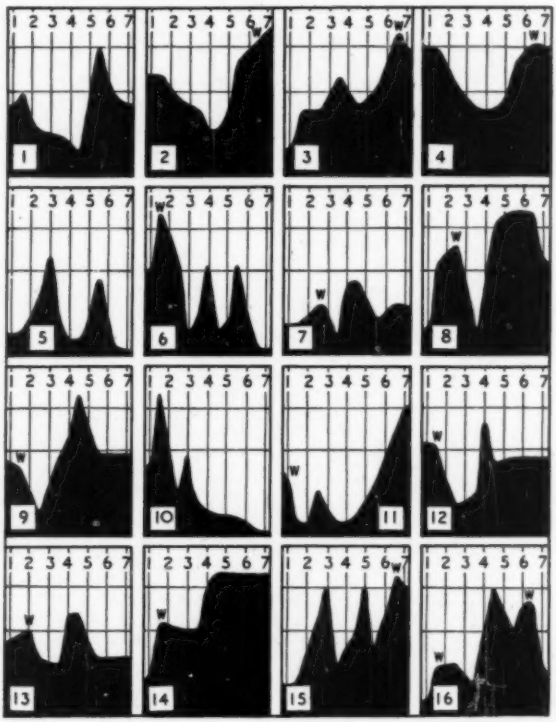


FIG. 2.

The "intensity" of a regional influence can be expressed in terms of the "degree of development" of patterns found within its zone of action; the more non-random the pattern the more powerful the influences at work.

The analysis is best shown graphically; the expected random frequency of the 7 grades and 6 inter-grades patterns of each map are plotted out on an inverted logarithmic scale so that "peaks" show high degrees of pattern development—that is, powerful regional influences.

The striking feature of the curves so formed is their essential simplicity. In fact they are almost as simple as the bold geographical zones from which they arise (Fig. 3 and 5). E.g. take No. 1; here we have a peak ($P = \cdot 01$) between grade 5 and 6 falling off on each side to grades 4 and 7. This shows the intensity structure of the black zone on map 1. There is also another peak ($P = \cdot 05$) between grade 1 and 2 falling off towards grade 4. This shows the intensity structure of the single hatched zone on map 1. Clearly, we have two unrelated zones of regional influences, one of which increased and the other of which decreased relative mortality. Moreover, *each zone represents a single influence.*

Again, Nos. 15 and 16 each show two separate peaks of positive regional influences corresponding to two separate black zones on each of maps 15 and 16, and so on. In general we may say that every peak represents a single distinct regional influence.

Peaks marked W show patterns in Welsh counties.

we combine this observation with that of the previous section, viz. that the intensity also follows definite *trends*, we begin to get a clearer picture of the essential attributes of these influences (Appendix 6).

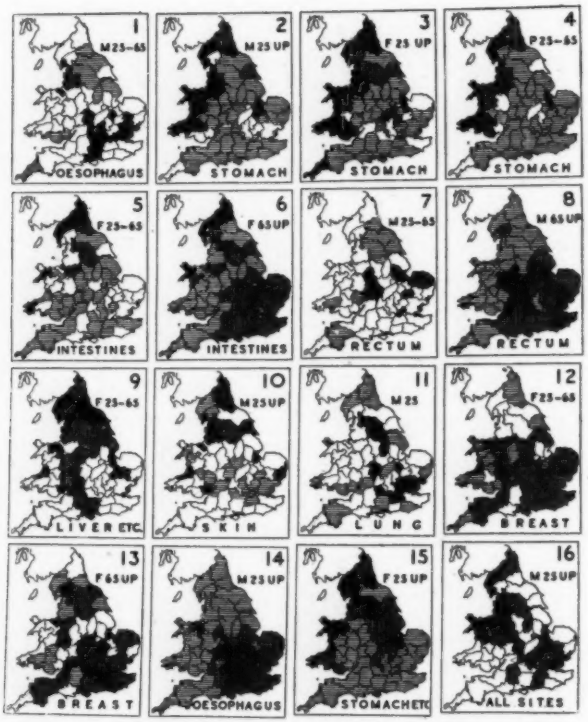


FIG. 3.

Every shaded county on these maps either participates in "significant" pattern formation or has a relative mortality showing "significant" deviation from the mean. They indicate the loci of regional influences. Generally, the shaded counties coalesce into "zones," which means that regional influences have bold and simple geographical distribution, and cover areas large in relation to the size of the single county units. This is a fundamental observation. Zones and counties are shaded in three ways; see Table below:

Shading.	Includes grades.	Effect of regional influences.
Black . . .	5 6 7 .	INCREASES Relative Mortality
Double hatch .	4 .	HOLDS at INTERMEDIATE level
Single hatch .	1 2 3 .	DECREASES Relative Mortality

Note particularly the important distinction between mere absence of a significant regional influence (unshaded) and the presence of regional influences actively decreasing mortality (single hatch).

The extent to which each zone represents a "single" regional influence is a matter for more detailed investigation; one method of approaching this problem is given in the adjoining footnote to Fig. 2.

VII. CORRELATION OF P TRENDS AND TOPOGRAPHICAL DISTRIBUTIONS.

In comparing corresponding curves and maps in Fig. 2 to 5 note:

1. That the shading of the zone varies with the position of the peak according to the following convention:

Peak at Grade 1, 2, or 3 shading is single hatching.
" " 4 " " cross hatching.
" " 5, 6, or 7 " " black.
Unshaded zones have $P > \cdot 05$.

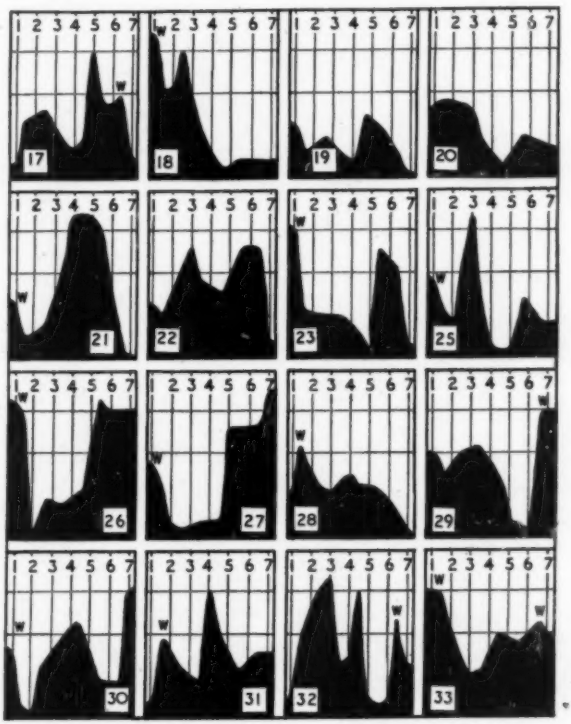


FIG. 4.
Legend as in Fig. 2.

2. That a significant " peak " on the curve ($P < \cdot 05$) is *always* associated with a significant zone on the map.

3. That a significant " zone " is *not always* associated with a significant peak. In such cases the individual counties of the zone each show a deviation from the mean $> \pm 2$ S.E. (Stocks' data), but collectively fail to participate in significant pattern formation. 1.5.7.10.19.20.25.28; usually such zones are associated with a " sub-critical " peak.

4. That significant peaks are not isolated, but rise naturally from less critically significant patterns of neighbouring grades. Even the low portions of the curves show strong evidence of non-random forms (hill and valley picture).

Correspondingly, significant zones on the map are not isolated, but merge naturally into zones of lower significance; unfortunately this feature only shows crudely on the maps, as zones are merely dichotomized into significant and non-significant. Fig. 6 gives a truer picture of the type of merging involved.

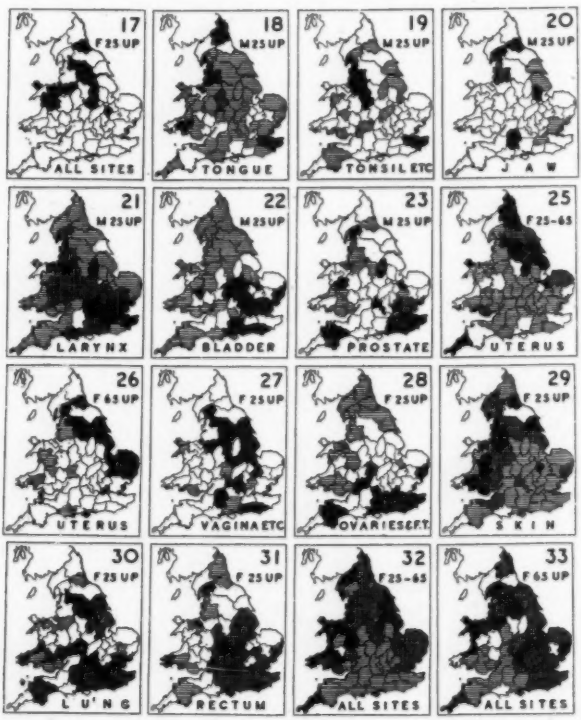


FIG. 5.
Legend as in Fig. 3.

5. That there are cases in which smooth trend of a curve is abruptly broken by some intermediate peak or minimum. These are generally associated with some degree of "isolation" or independence of the corresponding geographical zone (e.g. Welsh peak). 1.23.26.

6. That curves of type 2 which develop a peak before the extreme grades and then fall off are usually associated with maps in which the highest (or lowest) grades do not form a single zone, but break up into two or more sub-zones, e.g. grade 7 may appear as three separate zones, unrelated to each other, but all related to grade 6, ∴ pattern intergrade 67 very much more developed than the pattern of Grade 7.

7. That Wales is apparently the seat of very intense regional influences, for in 26 of the 32 curves there is a peak wholly or partially due to patterns in that country (marked W).

8. That where maps and curves relate to mixed data and not to a single form of cancer one may expect more complicated curves, as there is a greater likelihood of having multiple " centres " due to the superposition of several independent and unrelated pattern distribution. 15 (2–3) centres.

9. That often *apparently* simple geographical zoning (8, 22, 29, 31) is associated with quite complex curves. This apparent simplicity is due to the fact that Grade 5, 6 and 7 (or 1, 2 and 3) are not differentiated on our maps, and for more detailed study Dr. Stocks' originals should be consulted.

In comparing zones and peaks remember that a peak results from a " significant pattern," and that significant patterns arise from " geographical continuity of allied countries " ; and that this continuity results from a regional influence. Hence every peak represents a regional influence in some specific geographical zone.

VIII. THE GEOGRAPHICAL UBIQUITY OF REGIONAL INFLUENCES.

The zones of significance, which vary in position from map to map, indicate the loci of influences affecting various forms of cancer. How does the prevalence of these influences vary in different parts of the country ? In terms of the data available, this is equivalent to determining the *frequency* with which *each county* participates in significant pattern formation.

To ensure rough comparability in the data to be examined, we first select from the 32 maps analysed only those dealing with cancer of specific sites (e.g. we discard general categories such as " all forms "—maps 16, 17, 24, 32, 33).

The remaining 28 maps are then comparable in the sense that in each case we are using the relative cancer mortality for some particular site incidence as a test or index of the presence (or absence) of regional influences affecting that particular site.

Table VII shows, on this basis, the frequency (as a fraction of 28) with which each county participates in significant pattern formation, including single counties where the deviation exceeds $\pm$ 2 S.E. The frequency of effects " increasing " and " decreasing " the rate are shown separately ; the frequency of effects " modifying " the rate (significantly) is the sum of these two.

In the absence of regional influences we anticipate that a county will participate in " random " significant pattern formations in about 1/20 cases, i.e. 1·5 times per 28 county appearances. The *lowest* corresponding frequency recorded in Table VII is eight times greater than this (viz. Bedfordshire and Peterborough 12/28). The mean frequencies are, of course, still higher.

We therefore conclude (1) that these regional influences are geographically ubiquitous, and (2) that their prevalence (as measured by the number of forms of cancer they affect significantly) varies in different parts of the country.

IX. THE COMPOSITE REGIONAL INFLUENCE.

We have seen that while the topographical and intensity trends for individual site incidences are, in general, well defined (i.e. form a hill and valley picture), the zones of one map overlap to a varying degree the zones of another (Section VIII).

TABLE VII.—*The Number of Times (x) Each County Participates in Significant Pattern Formation.* (*Frequency* $= x/28$.)

County.	Effect of regional influence on relative mortality. Increases (I).	Decreases (D).	Modifies (I + D).	County.	Effect of regional influence on relative mortality. Increases (I).	Decreases (D).	Modifies (I + D).
Bedford	10	2	12	Staffordshire	9	8	17
Berkshire	10	9	19	Suffolk, East	10	7	17
Buckingham	11	8	19	Suffolk, West	9	7	16
Cambridge	10	7	17	Surrey	11	10	21
Cheshire	11	8	19	Sussex, East	11	10	21
Cornwall	2	11	13	Sussex, West	12	7	19
Cumberland	8	8	16	Warwick	13	10	23
Derby	4	12	16	Westmorland	13	8	21
Devon	7	13	20	Wight, Isle of	9	5	14
Dorset	3	9	12	Wiltshire	8	8	16
Durham	11	15	26	Worcester	9	9	18
Ely, Isle of	13	4	17	Yorks, E. Riding	11	10	21
Essex	12	8	20	Yorks, N. Riding	3	14	17
Gloucester	7	11	18	Yorks, W. Riding	13	9	22
Hereford	5	11	16				
				WALES.			
Hertford	9	9	18	Anglesey	6	8	14
Huntingdon	10	3	13	Brecknock	6	10	16
Kent	13	5	18	Caernarvon	6	10	16
Lancashire	12	10	22	Cardigan	8	9	17
Leicester	10	7	17	Carmarthen	6	13	19
Lincoln, Holland	13	5	18	Denbigh	9	8	17
Lincoln, Kesteven	9	9	18	Flint	15	4	19
Lincoln, Lindsey	10	12	22	Glamorgan	5	16	21
London	12	10	22	Merioneth	5	11	16
Middlesex	15	7	22	Monmouth	4	16	20
Norfolk	8	6	14	Montgomery	6	8	14
Northampton	10	6	16	Pembroke	7	7	14
Northumberland	10	9	19	Radnor	3	14	17
Nottingham	10	10	20				
Oxford	13	5	18				
Peterborough	9	3	12	MEAN	8·94	8·71	17·65
Rutland	8	6	14	S.D.	3·14	3·0	3·0
Shropshire	6	8	14				
Somerset	4	10	14				
Southampton	12	8	20				

This table includes single counties whose relative mortality deviates from the mean (= 100 per cent) by $\pm > 2 \times$ S.E. (Stocks' data).

Now, if the distributions and trends in each of the 28 maps are unrelated, the result of combining these inter-random distributions should be to flatten out the whole pattern (irregular multiple hillock picture). On the other hand, if they are related, their combination may be expected to result in some definite composite pattern or configuration (hill and valley picture).

From the entries of Table VII three contour maps were prepared (Appendix 7); these are shown in Fig. 6, and it is evident that whether we take the combinations of the distribution of regional influences, increasing, decreasing, or modifying the rates, we still retain well developed "hill and valley" effects.

This suggests that the various individual site patterns show some inter-relationship, or at least possess some " common factor."

(*N.B.*—The composite regional influence here discussed differs from the regional influence as measured in terms of relative mortality from " all causes." The latter deals only with the algebraic sum of plus and minus effects, while the present shows the magnitude of the sum of all effects, irrespective of sign.)

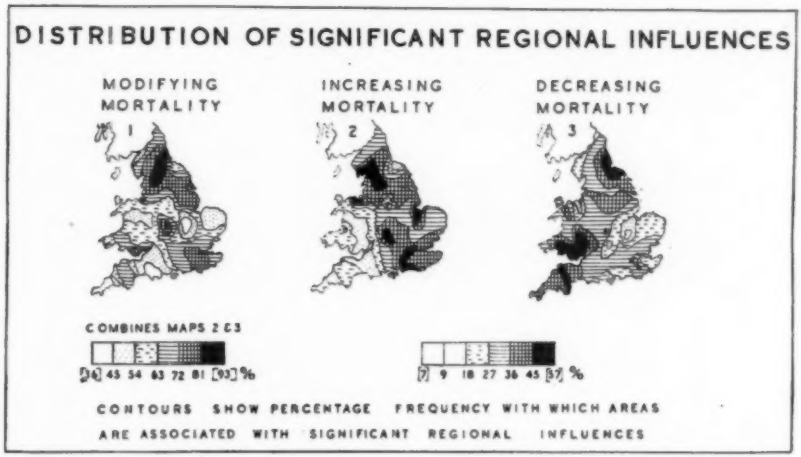

DISTRIBUTION OF SIGNIFICANT REGIONAL INFLUENCES
MODIFYING MORTALITY
INCREASING MORTALITY
DECREASING MORTALITY
1
2
3
COMBINES MAPS 2 & 3
36 45 54 63 72 81 [93] %
[7] 9 18 27 36 45 [57] %
CONTOURS SHOW PERCENTAGE FREQUENCY WITH WHICH AREAS ARE ASSOCIATED WITH SIGNIFICANT REGIONAL INFLUENCES

FIG. 6.

This shows the frequency with which regional influences are found in different parts of the country. It summarizes the data of Fig. 3 and 5, e.g. No. 3 sums all the " single hatched " counties and zones on maps 1–33, and indicates that significant influences (or zones) decreasing cancer are most frequent in the south-west and north-east.

Observe the ubiquity of regional influences, and also the general simplicity of their collective distribution.

Use of contours.—The area over which a regional influence acts is generally much more extensive than that of a single county (Fig. 3 and 5). Moreover, the intensity of pattern formation throughout this area follows a simple smooth trend (Fig. 2 and 4). We may therefore conclude that county boundaries have no essential significance in relation to regional influences. In fact these influences behave in every way as continuous variables, and their variations in intensity are most appropriately depicted by means of contour-lines on the surface of the map.

To depict these regional influences by contours may serve to drive home another of their characteristics as revealed by this study, viz. that they behave in every respect as " continuous variables." Any concept of discontinuity is an artefact arising from the use of " counties " as pattern units ; county boundaries have no essential significance in relation to regional influences.

X. THE GENERAL CHARACTERISTICS OF REGIONAL INFLUENCES.

These may now, on the basis of the preceding sections, be summarized as follows :

They are present to such an extent that between 1/5th and 1/3rd of all " patterns " are individually significant, and the collective mean probability of all patterns, including the non-significant, differs from random by at least 9 to

TABLE VIII.—*Effective* P *of Size of Group* (Pnn_1). *Gives the Probability of Any Selected Single Group Observed on the Map.* (N.B.—*Grade Pattern*

Number of counties in Grade = *n*.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.
2 .	100	8			.					.					.		
3 .	100	19	1		.					.					.		
4 .	100	38	5	*	.					.					.		
5 .	100	63	7	1	*					.					.		
6 .	100	89	18	6	*	*				.					.		
7 .	100	100	31	10	1	*	*			.					.		
8 .	100	100	47	15	4	*	*	*		.					.		
9 .	100	100	59	22	7	2	*	*	*	.					.		
10 .	100	100	72	31	16	6	1	*	*	*					.		
11 .	100	100	89	43	24	9	2	1	1	*	*				.		
12 .	100	100	100	52	30	12	4	1	1	1	*	*			.		
13 .	100	100	100	64	37	16	7	4	2	1	*	*	*		.		
14 .	100	100	100	77	48	21	9	6	4	3	2	1	*	*	.		
15 .	100	100	100	93	56	28	17	10	6	4	3	2	1	*	*		
16 .	100	100	100	100	69	38	25	18	11	5	4	3	2	1	*	*	
17 .	100	100	100	100	82	43	30	24	18	11	5	4	2	1	*	*	*
18 .	100	100	100	100	91	53	38	29	24	17	11	6	5	3	1	*	*
19 .	100	100	100	100	100	70	48	35	30	23	14	9	6	4	2	1	*
20 .	100	100	100	100	100	80	59	41	34	28	19	14	10	7	4	2	*
21 .	100	100	100	100	100	93	66	47	41	34	26	21	17	13	10	6	3
22 .	100	100	100	100	100	100	81	65	54	43	33	26	22	18	13	8	4
23 .	X	X	X	X	X	X	X	X	X	X	45	37	28	23	19	15	10
24 .	X	X	X	X	X	X	X	X	X	X	X	40	30	24	19	16	12
25 .	X	X	X	X	X	X	X	X	X	X	X	X	40	33	26	20	15
26 .	X	X	X	X	X	X	X	X	X	X	X	X	X	40	31	24	20
27 .	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	30
28 .	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
29 .	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
30 .	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	33
31 .	X	X	X	X	X	X	X	X	X	X	X	X	X	X	39	39	39
32 .	X	X	X	X	X	X	X	X	X	X	X	63	63	63	63	63	63
33 .	X	X	X	X	X	X	X	X	X	66	66	66	66	66	66	66	66
34 .	100	100	100	100	100	89	81	73	67	67	67	67	67	67	67	67	67
35 .	100	100	100	100	90	79	70	64	64	64	64	64	64	64	64	64	64
36 .	100	100	100	89	81	69	60	60	60	60	60	60	60	60	60	60	60
37 .	100	100	100	80	72	64	57	57	57	57	57	57	57	57	57	57	57
38 .	100	100	100	78	71	61	54	54	54	54	54	54	54	54	54	54	54
39 .	100	100	96	74	66	58	51	51	51	51	51	51	51	51	51	51	51
40 .	100	100	73	53	46	39	33	33	33	33	33	33	33	33	33	33	33

Size of

To find the effective prob
1. Divide tabulate
2. Call "X" $P = <.01$
3. Call "*" $P = >.5$

18 times the S.E. Significant patterns can be demonstrated in all parts of England and Wales, their *minimum* presence (or frequency) being still eight times greater than random.

The intensity of their effect usually varies in a simple and regular fashion both between consecutive grades and across contiguous geographical areas ; i.e. they behave as " continuous variables " and their effects are most appropriately represented on maps by means of contour lines.

As operative on any particular form of cancer regional influences appear to be few rather than many in number and developed over wide rather than over small tracts.

Finally, although the regional influences affecting different forms of cancer each have their own distribution, all are apparently related to some common key

Obtaining, by Chance, a Single Group of Counties Equal to or Larger in Size *than can be Assessed in Terms of the* Pnn_1 *of the Largest Single Group Present.)*

Group = n_1.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.	40.

obtaining, by pure chance, a group of n_1 *or larger*; and reference to Table IX under arguments n and g will give the probability Png that the n counties are arranging themselves, by pure chance, *into g or some smaller* number of groups.

TABLE IX.—*Effective* P *of Number of Groups* (Png). *Gives the Probability that the Counties in Any Selected Grade will Arrange themselves by Chance into an Equal or Smaller* Number *of Groups than Observed on the Map.*

Number of counties = "n."	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
	Number of Groups in the Grade = "g."														
2	8	100													
3	1	19	100												
4	*	5	38	100											
5	*	2	11	58	100										
6	*	1	6	34	71	100									
7	*	1	2	18	52	82	100								
8	*	*	3	13	34	66	91	100							
9	*	*	3	7	29	52	78	94	100						
10	*	*	2	7	22	44	67	89	98	100					
11	*	*	2	4	14	35	62	83	96	100	100				
12	*	*	2	3	15	27	53	77	92	98	100	100			
13	*	*	2	5	13	25	46	74	83	94	98	100	100		
14	*	*	1	3	10	25	51	67	80	89	97	99	100	100	
15	*	*	2	4	6	22	47	62	79	88	96	99	100	100	100
16	*	*	2	4	9	22	47	63	78	90	95	99	100	100	100
17	*	*	2	5	11	21	45	68	77	87	93	99	100	100	100
18	*	*	2	4	13	23	40	63	76	87	95	98	100	100	100
19	*	*	1	3	12	22	44	61	79	87	94	100	100	100	100
20	*	*	1	3	11	25	46	60	80	88	94	99	100	100	100
21	*	*	1	3	10	25	48	69	83	94	97	98	99	100	100
22	*	*	1	4	13	27	46	71	82	94	97	99	99	100	100
23	*	*	2	7	16	29	55	70	88	96	97	99	99	99	100
24	*	*	1	5	13	34	57	74	89	97	98	98	100	100	100
25	*	*	3	8	19	40	60	74	92	97	98	98	100	100	100
26	*	*	3	7	24	45	60	81	92	96	97	99	99	100	100
27	*	1	4	14	29	51	67	82	94	96	96	99	100	100	100
28	*	2	6	19	33	55	72	87	95	96	98	100	100	100	100
29	1	2	8	21	35	60	80	87	96	97	98	100	100	100	100
30	1	2	9	22	38	69	84	90	96	97	98	100	100	100	100
31	2	5	12	26	44	75	88	91	97	98	99	99	100	100	100
32	2	5	15	37	59	80	91	94	96	97	99	99	100	100	
33	3	7	26	45	67	83	91	95	97	98	100	100	100		
34	4	9	27	49	73	89	93	96	98	100	100	100			
35	4	11	29	55	77	86	93	95	99	100	100				
36	4	17	38	60	82	91	96	98	100	100					
37	5	17	41	64	85	96	98	100	100						
38	4	22	46	69	89	95	99	100							
39	6	26	52	77	90	99	100								
40	7	29	58	83	95	100									

To find the effective probability of the number of groups Png—
1. Divide the tabulated value by 100.
2. Call * $P = < \cdot 01$.

The analysis is entirely automatic (see Appendix 2), and no judgment is required other than deciding whether or not counties are actually "in contact," so forming "a group." This is usually perfectly obvious, but there are a few

doubtful instances which are dealt with by the arbitrary standardization shown in Fig. 7.

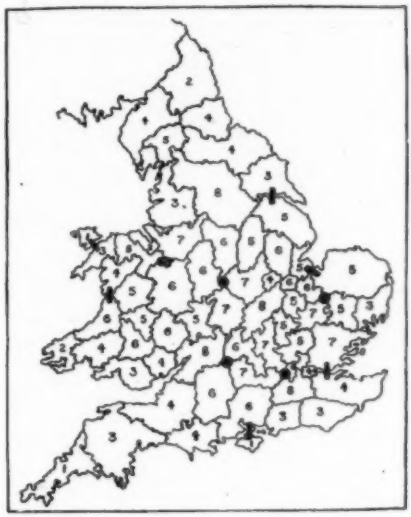


FIG. 7.

This map shows how the " contact number " varies from county to county, e.g. for Devon this is 3 as the county " contacts " three others, viz. Cornwall, Somerset and Dorset. While the majority of counties meet at a common county boundary, occasionally they meet only over a very narrow range, the so-called " point contacts " (solid circles on map). Where the county boundary is a river or an estuary the counties may fail to make a *de facto* contact, and yet, it may be necessary to consider these as " touching " for the purpose of studying regional influences (solid rectangles on map).

Note the distinctly higher values of the contact numbers of the inland counties as contrasted with those of the coastal counties.

The mean contact number (C) works out at 5·12. This is an important constant in theoretical formulae, e.g. the expectation of obtaining " pairs " with $\frac{n}{N}$ counties occupied is $\frac{C(n \times n-1)}{2(N-1)}$ and so on.

APPENDIX 2.

MECHANICAL ANALYSIS OF PATTERNS.

A " mirror-outline " county map is drawn with a ""glass-ink " on the undersurface of a sheet of glass. Invert and place upon and congruent with map to be analysed. Mark on the glass with grease pencil all " Grade 1 " counties. Remove glass template and place on white sheet. Analyse arrangement of marked counties ; record ; clean off wax pencil mark with chloroform. Replace on map and proceed similarly to work and analyse " Grade 2 " counties, and so on. By this means grades are presented for examination free of confusing context. It is helpful to indicate permanently on the template the arbitrary decisions *re* contacts, i.e. to make it look in all regards as Fig. 7.

Intergrading is carried out by appropriate modification of the above procedure. Records are entered on the special analysis sheets (Appendix 3).

APPENDIX 3.

RECORDING PATTERNS.

Sample Analysis Sheet (Map No. 17).

Grade. Number	Number in "n"	Size of groups "n"$_1$. 1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	>14.	Number of Groups "g."
1	2	//	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
2	10	////	/	..	/	..	..	..	..	..	..	..	..	..	..	..	6
3	20	///	..	/	..	/	..	..	..	/	..	..	..	..	..	..	6
4	19	//	//	//	..	..	..	/	..	..	..	..	..	..	..	..	7
5	17	//	..	..	..	/	..	..	..	..	..	..	..	..	..	..	3
6	2	..	/	..	..	..	..	..	..	..	..	..	..	..	..	..	1
7	2	//	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
	62	15	4	3	1	2	*	1	*	1	*	*	*	*	*	*	27

Check $\Sigma n = 62$ (always). Σ columns + Σ rows + 27 (in this case):

$(15 \times 1) + (4 \times 2) + (3 \times 3) + (1 \times 4) + (2 \times 5) + (1 \times 7) + (1 \times 9) = 62$ (always.)

The P values corresponding to the various ng (and/or nn_1) entries are read off from Tables IX (and/or VIII) and finally collected in a summary sheet.

Sample Summary Sheet (Map No. 17).

Grade.	1.	2.	3.	4.	5.	6.	7.	
ng	2;2	10;6	20;6	19;7	7;3	2;1	2;2	(*)
Png	*1·00*	*·44*	*·25*	*·44*	*·02*	*·08*	*1·00*	
nn_1	2;1	10;4	20;9	19;7	7;5	2;2	2;1	
Pnn_1 (if $< Png$)	..	..	(·31)	..	(01)	..	..	(†)

(*) i.e. Second and final column entries of analysis sheet.

(†) i.e. Second column and highest recorded value of corresponding n_1.

Intergrade analyses are entered on similar sheets.

APPENDIX 4.

HIGHER PATTERNING OF INTERGRADES.

The 192 intergrades are composed entirely of the 210 grades. Each intergrade, being composed of two grades, is, on the average, just twice the size of the individual constituent grades. The patterns in these two units are analysed by a method which fully compensates for difference in size. Hence, we might anticipate that the means for these two " sampling " series (i.e. of the 192 intergrade- and 210 grade-patterns) *should* show good agreement. That this is not the case calls for some explanation.

The map pattern contains 62 elements (counties). To formalize take the analogous special case of an 8×8 " map " with the basic pattern shown in Fig. 8. Then the random probability of any *fragment* (or grade) of this pattern is greater than that of a larger fragment (or intergrade) and still greater than that of the whole, e.g.—

1/4 fragment (16 squares) $\quad P_{16} = \dfrac{13!\ 3!}{16!} = \cdot 1785 \times 10^{-2}$

1/2 fragment (32 squares) $\quad P_{32} = \dfrac{26!\ 6!}{32!} = \cdot 1104 \times 10^{-5}$

whole (64 squares) $\quad P_{64} = \dfrac{52!\ 12!}{64!} = \cdot 1269 \times 10^{-12}$

and $P_{16} > P_{32} > P_{64}$.

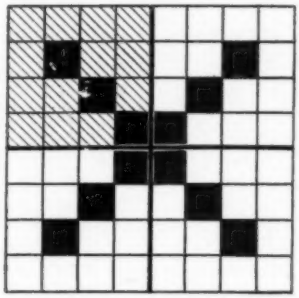


FIG. 8.

It is an interesting fact that the *larger* the area we select for examination the *higher* is the degree of patterning found. As this is not due to the method, which fully compensates for variations in the size of the area examined, it must be attributed to some intrinsic feature of the Regional Influences.

Fig. 8, in conjunction with Appendix 4, indicates the probable, simple explanation, viz. that estimates of the degree of pattern development of an *organized* pattern must generally fall below the true value when they are based on the examination of only part of the pattern—for the simple reason that the relation of one " piece " of the pattern to a neighbouring " piece " is thereby overlooked. The process of " intergrading " was evolved to deal with this contingency.

Thus, to explain the observed fact that the P grade greater than P intergrade we must assume that the mean " pattern " associated with regional influences covers an area greater than that of a mean grade, i.e. greater than 9 counties $(\frac{62}{7})$. In terms of area this means >940 sq. miles.

APPENDIX 5.

SIGNIFICANT DEVIATIONS OF RELATIVE MORTALITY.

Where, for example, there is only one county in a grade (or intergrade) pattern formation becomes impossible and Png and Pnn_1 must both $= 1{\cdot}00$; such cases are *italicized* in Table I. But the relative mortality for such a " single " county may deviate so markedly from the mean (i.e. exceed 2 S.E.) that regional influences may be assumed operative. Hence, in cases where a qualitative result only is required (i.e. significant regional influence " present " or " absent ") it is natural to use this supplementary—and orthodox—source of information. It is readily obtainable, as Dr. Stocks classifies all his data with reference to deviations from S.E., viz. as being 0–1, 1–2, and 2 + times S.E.

APPENDIX 6.

VISUALIZING COMBINED DATA OF SECTIONS V AND VI.

These zones of significant patterning are not " isolated," but merge with zones of high, though not significant, pattern. These in turn merge with zones of lower pattern. That is, they show geographical trends.

The easiest way to integrate the P trends of Section III and the geographical trends of Section IV is to start with a two-dimensional graded map and imagine the level of each county raised in proportion to the reciprocal of the P values for

the grade (and/or intergrade) to which it belongs. This will give a three-dimensional topographical map, whose surface undulations reflect variations in pattern intensity (i.e. by previous definition, variations in the intensity of regional influences), and whose surface shadings show the distribution of the grades.

When we have made this integration we find that the surface of the map is developed into a few well-marked hills and valleys, the " peaks " of these hills representing areas of " significant " patterning.

APPENDIX 7.

PREPARING CONTOUR MAPS.

A county map was pasted on a board. Metal pins, corresponding in height to the frequencies in Table VII were driven vertically into the centre of each county; the whole was then built up with plasticine to the level of the top of the pins. A scriber-gauge, adjustable to various heights, was attached to a pantograph. With this apparatus the contours of the three-dimensional model were automatically transferred to the two-dimensional map-surface.

APPENDIX 8.

NEGATIVE REGIONAL INFLUENCES.

There is definite evidence that " negative " influences, i.e. regional influences decreasing cancer exist alongside the " positive," in fact these two types of influence, both of which are represented by peaks and zones, occur with equal frequency (Table VII). Negative effects must not be regarded as merely due to the absence of positive effects, for the observed type of trend and degree of patterning associated with negative zones is of a much higher order than is to be expected from the mere absence of positive effect.

It seems that there must be some general factor *other than regional* which operates to produce a definite incidence of cancer. Regional influences are not themselves the cause of cancer, but merely impress their effect, positively or negatively, upon this general factor.

I wish to express my thanks to Mr. N. R. Ling for preparing the various diagrams.

REFERENCE.

CRUICKSHANK, D. B.—(1940) *Papworth Res. Bull.*, p. 36.

CARCINOMA OF THE BREAST. AN ANALYSIS OF THE SYMPTOMS, FACTORS AFFECTING PROGNOSIS, RESULTS OF TREATMENT AND RECURRENCES IN 1211 CASES TREATED AT THE MIDDLESEX HOSPITAL.

B. McN. TRUSCOTT.*

From the Middlesex Hospital, London, W.1.

Received for publication March 25, 1947.

THIS investigation was undertaken to bring up to date the records of the Middlesex Hospital, and to attempt to discover whether there was any statistical evidence to support commonly held views on factors affecting prognosis and methods of treatment. 836 cases of carcinoma of the female breast treated during the years 1926–35 were analysed and a smaller group of 265 cases, treated during the years 1936–40, were the subject of a further investigation to discover the effect of more modern methods of treatment.

The hopes held at the start of this work have not been completely fulfilled. For such an investigation to give a clear cut statistical answer to all of the controversial questions, careful planning and equal distribution of cases, according to the extent of the disease and type of treatment used, would be necessary. This was not so in this series of cases. As a result the evidence on some points is too inconclusive to give an answer.

Careful analysis of results would seem to be essential if methods of treatment are to be guided by fact rather than by impressions. If, in the future, reliable figures on a large scale are to be obtained, co-ordination of policy in treatment and planning ahead in large medical formations will be necessary. Whether this is acceptable or desirable may be open to argument, for it must inevitably mean some acceptance by each individual surgeon of the combined opinion of his colleagues. Only thus, however, can the successful analysis of large series be substituted for the analysis of smaller series for individual surgeons.

Despite the difficulties that have arisen owing to the many variations in technique during the years covering this investigation, certain facts that may be of interest and value have emerged. Some previously held views have been supported; others appear to be incorrect.

The histological findings in this series have been omitted owing to the impracticability of examining over 1000 sections in the time available. This in itself would make the basis of a separate inquiry. From the statistical point of view therefore it has been assumed that any variations in the histological grouping of the growths have been spread evenly throughout the series.

The presentation of results of an investigation such as this is difficult. While the actual detailed figures and tables by which conclusions have been reached are of importance, their inclusion in the text would make reading wearisome. Therefore simplified tables only have been included.

* Late Surgical Registrar (demobilized), Middlesex Hospital.

The investigation has been made on the following plan:

1. The fate of 836 cases after ten years.
2. The influence of factors on this result:
   (*a*) The clinical state of the growth at the start of treatment.
   (*b*) The site of the growth in the breast.
   (*c*) The age of the patient.
3. The treatments employed and their results.
4. Symptomatology.
5. Recurrences, their distribution and relation to the site of the primary growth.
6. The 10–15-year period after treatment.

Method of investigation.

Material.

All cases that came for treatment during 1926–35 have been included with the following exceptions: some whose records could not be found; some on whom there was insufficient information; some who were considered too far advanced for any treatment; some operated on elsewhere and sent for prophylactic radiotherapy or for treatment of recurrences.

Collection of data and cases.

All information on the original condition and the treatment has been extracted from the notes made at that time. Subsequent progress has been followed from records kept by the hospital follow-up department. Where there has been a long interval since the last visit or letter, an attempt has been made to complete the information by writing to the patient. This has been successful in a fair proportion of cases, but there are many that seem to be untraceable since their evacuation or forcible eviction by enemy action during the war.

Deaths.

Information as to the cause of death is largely from the general practitioner. Post-mortem examinations were not done on the majority, and the recorded cause of death may not always be completely accurate. It would seem unlikely that this small potential error has affected the results to any marked degree.

Recording of result and length of follow-up.

One of the following results was possible for each patient at the end of five, ten and fifteen years: alive and well; alive with a recurrence; untraced; dead.

If dead the cause of death could be due to carcinoma of the breast, to other causes or to unknown causes.

The length of follow-up was until July, 1946, so the maximum was twenty years and the minimum was ten years. For the purpose of comparing factors affecting the result and to conform with other published series two time intervals have been used—the 5 years and 10 years from treatment. While the results at the end of fifteen years were of considerable interest, they were not sufficiently consistent in follow-up to be used for comparisons.

Method of expressing result.

Recbrding of the result according to the six possible fates previously mentioned is too cumbersome to allow easy assessment of the effects of varying factors. Those alive at the end of each period are successes and those dying of carcinoma are failures, but those in between fall into neither class. A case dying of other causes or of unknown causes cannot really be called a success or a failure.

Two ways of expressing the result seem to be possible—the survival rate or the mortality rate. The former has the advantage that it allows easier comparison with previous series that are almost universally expressed in terms of survival (or cure) rates. It would appear, however, that results expressed in terms of those actually dying of carcinoma of the breast will give a more accurate picture of effects of various factors influencing the prognosis.

For these reasons the " mortality rate " has been used as a routine in this paper. It has been expressed as the *percentage of those cases dead from carcinoma of the breast of all traced cases.* The survival rate has also been recorded, as it may be of interest for comparison with other series.

1. *The Fate of* 836 *Cases of Carcinoma of the Breast Ten Years after Treatment.*

The following table shows the end-result of all stages of carcinoma of the breast after treatment :

Result.	Number.
Alive	94 (11·0 per cent)
Dead :	
From operation	20 (2·5 ,,)
,, carc. mammae . . .	513 (61·5 ,,)
,, other cause . . .	62 (8·0 ,,)
,, unknown cause . . .	48 (6·0 ,,)
Untraced	99 (11·0 ,,)
	836

The mortality rate from carcinoma of the breast is therefore 533 cases out of 737 traced cases, or 72·3 per cent, and the survival rate is 94 out of 737 traced cases, or 12·75 per cent. This mortality rate means that three out of every four women coming for treatment during these years died of carcinoma of the breast within ten years. These figures can only have a disturbing effect on any feeling of satisfaction or complacency with the treatment of carcinoma in an area that has been recognized as one of the most approachable.

2. *The Influence of Factors on the Result.*

a. *The clinical state of the growth at the start of treatment.*

Each case was put into stages according to the extent of the growth at the start of treatment.

The following stages were used :

Stage I.—Growth in breast *not* infiltrating skin or muscle, *not* involving axillary glands, no other metastasis. (Slight attachment to skin or muscle was not considered as infiltration.)

Stage II.—Growth as above but *with involvement of axillary glands.* This involvement was on histological grounds if possible, but in cases where this had not been done clinical enlargement was taken as involvement.

Stage III.—(*a*) Growth in breast with skin infiltration or ulceration *without* axillary gland involvement.

(*b*) Growth in breast with skin infiltration or ulceration *with* axillary gland involvement.

Stage IV.—As in Stage I–III, but with distant metastasis, supraclavicular glands to count as distant metastasis.

The results of all treatment were determined for each stage of growth. The simplified tables read as follows:

Mortality Rate at Five Years and Ten Years.

Stage of growth.	Percentage mortality rate.	
	5 years.	10 years.
I	36·2	53·0
II	69·0	75·0
III*a*	73·7	73·7
III*b*	88·2	93·5

The striking feature about these tables is the marked difference between the results for Stage I and Stage II growths. There is a 33 per cent difference in the mortality rate at five years if the axillary glands show involvement. The standard error of difference is 3·6 per cent; the measured difference is more than eight times this and is, therefore, highly significant. These figures bear out the reports of others as to the great importance on prognosis of examination of the axillary glands histologically (Scarff and Handley, 1938; Leeds Report, 1926).

The figures for Stage III growths are based on a small number of cases, but they suggest that ulceration of the skin even when the axillary glands are free from growth is of bad prognosis. When both ulceration and axillary gland involvement are present as in Stage III*b* cases the outlook is gloomy in the extreme.

The survival rate for Stage I cases is 51·4 per cent. This appears poor when compared with other series, but it should be remembered that this figure includes only those cases alive. Any case dying of unknown cause or other cause in this method of calculating the survival rate would be counted as a failure. If such cases are excluded from the total the figure would be very close to 60 per cent.

A point that is striking is the number of cases that die from a carcinoma of the breast during the 5–10-year period. There is a consistent increase in mortality rates at the ten-year interval for all stages of growth. In Stage I cases 75 patients died during the first five years and a further 24 in the second five years. Again this was pointed out by Scarff and Handley in their paper. In this investigation it has been found that there is even a mortality after ten years. In 250 Stage I cases a further 6 died between the 10th and 15th years. This point is further elaborated in Section 6 of this paper.

Further analysis of Stage I cases into the effect of slight attachment to skin or deep tissues.

Although all Stage I cases were free from infiltration of the skin or deep tissues, some of them had a certain degree of attachment or "tethering." To

determine whether or not this was of importance in the prognosis, a further subdivision was made into cases *with* early attachment and cases *without* attachment and the mortality rate determined for each.

There were 125 of each type and the mortality rates were as follows :

Mortality Rates at Five and Ten Years.

Stage.	Percentage 5 years.	Percentage 10 years.
I, no attachment	31	51
I, slight attachment	38	55

These differences are too small to be of statistical significance in this number of cases, but the table suggests that attachment had an adverse effect on the prognosis. A further subdivision was made of cases with slight attachment into those with attachment to the skin and those with attachment to deeper tissues. The mortality rate for these sub-groups was :

Stage.	Percentage 5 years.	Percentage 10 years.
Deep attachment	47	58
Skin attachment	33	53·5

It will be noticed that the mortality rate for cases with slight attachment to the skin is very similar to those with no attachment at all, 33 per cent and 31 per cent, while that for cases with slight attachment to the deep structures is considerably higher, 47 per cent. On this number of cases the difference is barely significant, but it would suggest that early attachment to the deep tissues is of serious import, while slight attachment to the skin does not materially affect the prognosis.

b. *The site of the growth in the breast.*

In describing the situation of a growth in the breast the usual subdivision into quadrants was made. When a growth lay between two quadrants it was described as lying in a " mid " position. Thus there were the " mid-medial," the " mid-inferior," the " mid-lateral " and the " mid-superior " positions. A growth lying immediately beneath the nipple or spreading into three or more quadrants was described as being in the " central " position. A growth lying in the axillary tail was put under that heading. Tables showing the distribution in all these positions are too cumbersome, and they have been simplified by dividing growths in the mid-positions equally between the adjacent quadrants.

The distribution of growths among the quadrants was similar in Stage I and Stage II growths and the consolidated tables are :

Quadrant.	Percentage of growths.
Outer and upper	46
Inner and upper	20
Central	13
Outer and lower	12
Inner and lower	5
Axillary tail	4

The rather high incidence of growths in the central position is probably due to a certain number of larger growths in Stage II that were not described in sufficient detail to put them into any other quadrant.

To attempt to discover whether there was any difference in the prognosis between the various sites, the mortality rate for each site for Stage I and II growths was determined :

Stage I and II Growths. Mortality Rate at 5 Years for Each Site.

Site.	Stage I growths. Percentage mortality rate 5 years.	Stage II growths. Percentage mortality rate 5 years.
Outer and upper	33·5	62·0
Inner and upper	38·5	67·0
Central	37·0	78·0
Outer and lower	31·5	69·5
Inner and lower	28·5	58·5
Axillary tail	33·5	73·5

These figures do not show any consistent difference, and there is little to suggest that the site of the growth in the breast plays an important part in prognosis. It may be worthy of note that the inner and lower quadrant has the lowest mortality rate in both Stage I and Stage II growths, being at least 10 per cent lower than the highest in each group. This agrees with the findings of Perry (1925) in his series from the London Hospital.

c. *The age of the patient.*

The average age of the patients in this series was 55 years. The average age for Stage I growths was 55 years, for Stage II growths 54 years, and for Stage III growths 59 years.

The incidence of growths in decades was :

Decade.	Percentage.
0–39	8·0
40–49	26·5
50–59	30·0
60–69	24·0
70–79	10·0
80–89	1·5

It is remarkable that the youngest was a girl of eleven, alive and well fifteen years later. The oldest was a woman of 86.

To determine the effect of age on prognosis the mortality rate was calculated for each stage and for each decade.

The consolidated table shows the following result:

Decade.	Percentage mortality rates. 5 years.	10 years.
0–39	70	81
40–49	59	74
50–59	63	76
60–69	62	79
70–79	56	63
80–89	67	67

Once again these figures are somewhat disappointing in that they show little difference between the age groups. The decade 80–89 is a group of 13 cases only and must be disregarded. Although the mortality rate for group 0–39 is higher than any other, both for the consolidated table and for each stage separately, the difference is not so striking as might be expected. There is no support for the opinion that a carcinoma of the breast in a young woman is invariably fatal. The prognosis in Stage I growths is little worse than for the other age groups.

3. *The Treatments Employed and Their Results.*

Methods employed.

All the cases in this series have been treated by the Honorary Surgical Staff of the Middlesex Hospital during the years 1926–35. The varying methods used were given according to the wishes and opinions of those surgeons.

Three main types of treatment were used: surgery alone; surgery and radiotherapy; radiotherapy alone.

In view of the findings in this part of the investigation it should be stressed that the technique of radiotherapy was in the process of evolution. Indeed, there was an abrupt change in 1936, and the further investigation on cases treated in 1936–40 modifies the results obtained in this series.

Radiotherapy, when used alone, was either radium implant to the breast in the Keynes (1932) technique or deep X-ray therapy. When associated with surgery the radiotherapy was either the implantation of radium into the wound at the time of operation according to the method described by Sampson Handley (1922) or prophylactic deep X-ray therapy. The latter was given in multiple intermittent sub-erythemal doses with medium voltage. The dose was recorded in "P.D." or "percentage E.D." and appeared to vary considerably. The treatment was carried out for prolonged periods occasionally extending up to three years, and on several occasoins the prophylactic therapy merged imperceptibly into treatment of a local recurrence.

Selection of method of treatment.

If any comparison is to be made of the results of these differing methods of treatment, one must be sure that there was no tendency to use one method on the more unfavourable case. It is difficult to assess from the notes why various methods were used, but one may assume that the standard of skill over the whole series was equal. It would seem possible that there was a tendency to use radio-

therapy on the more extensive case, but this was not by any means invariable. Some surgeons never used it on any case, no matter how severe, while, in another large series, radium was employed as a routine at operation.

The group treated by radiotherapy alone were those with concomitant disease that made them unfit for surgery: those that refused operation and those that were considered unsuitable for operation, usually owing to the small size of the breast. There would therefore seem to be a case for saying that this group might be expected to show worse results than the others.

Comparison of results.

In order to gain a common ground for comparison the results have been determined for each method of treatment on Stage I and Stage II cases. In addition, to determine the results in a completely homogeneous early group, the same figures have been calculated for the earliest type of Stage I case, i.e. those with no attachment at all.

To simplify the results and to obtain groups of sufficient size for satisfactory comparison, cases that received any form of radiotherapy as an aid to surgery have been put under one heading.

Results of Treatment.

Stage I growths.

Method of treatment.	Percentage mortality 5 years.	Percentage mortality 10 years.
Surgery alone	28	49
Surgery and radiotherapy . .	47	63
Radiotherapy alone . . .	43	86

Stage II growths.

Surgery alone	62	72
Surgery and radiotherapy . .	74·5	82
Radiotherapy alone . . .	70	73

The striking feature about these tables is the marked increase in mortality rate in those cases that received ancillary radiotherapy to surgery over those treated by surgery alone. In Stage I there is a 19 per cent increase at five years, which is well over twice the standard error of difference (7 per cent). Similar findings will be seen for Stage II growths.

What interpretation is to be put on these figures? At their face value it would seem that cases treated with ancillary radiotherapy have a significantly higher mortality rate, and, to put it bluntly, the use of radiotherapy was actually harmful. This disconcerting result cannot be accepted unless the cases treated by all methods were homogeneous. As stated above, there may have been a tendency to send the worse cases for radiotherapy, but it can only have a slight effect on the result.

To exclude this tendency as far as possible the same mortality rate has been calculated for the earliest type of Stage I cases. These will be cases with a good prognosis and equal on clinical grounds. This group gave the following figures:

Stage I, with No Attachment at all.

Method of treatment.	Percentage mortality 5 years.	Percentage mortality 10 years.
Surgery alone . . .	25·5	45·5
Surgery and radiotherapy .	43	58·5
Radiotherapy alone . .	20	54·5

There were only 125 cases in this class and figures are too small to be of statistical significance in themselves, but it will be noted that they are in the same ratio for the figures for Stage I growths as a whole with regard to surgery and surgery with radiotherapy. The series treated by radiotherapy alone was far too small to be of any significance. It is therefore reasonable to infer that these figures show a true difference in mortalities.

To sum up this evidence, it seems strongly suggested that the results of treatment of carcinoma of the breast were best, during the years 1926-35, when surgery only was the method of treatment.

Subsidiary investigation into results of treatment during the years 1936–40.

In the year 1936 a big change took place in the technique of radiotherapy when used with surgery. Instead of multiple sub-erythemal doses given over a prolonged period, the treatment was given in daily doses up to the level of skin tolerance, using 250 k.v. Once this level had been reached prophylactic treatment was not repeated. The average dosage was 2400 " r " to the skin over four fields, with a tumour dose of approximately 3500 " r " to the chest wall and the glands.

This subsidiary investigation has been conducted to try and determine whether the results improved with the change. If any practical value as to the best method of treatment is to be gained it is obvious that the treatments compared must be as modern as possible. Unfortunately, owing to the war, the information is not as full nor the follow-up as complete as might be wished.

The following mortality rates at five years for Stage I and Stage II growths treated by surgery and radiotherapy were found :

Stage I : 5-year mortality rate, 24 per cent.
Stage II : 5-year mortality rate, 70 per cent.

When these figures are compared with similar figures for the previous period (47 per cent and 74·5 per cent) it will be seen that there has been considerable improvement in Stage I and slight improvement in Stage II growths. When comparing these figures with those achieved by surgery alone the figures for Stage I growths are slightly better and those for Stage II growths are slightly worse when radiotherapy was added to surgery. Neither of these differences is, however, large enough to be significant statistically.

To summarize the whole position, it may be said that the change of method of administering radiotherapy since 1936 has resulted in an improvement in the results achieved by the combination of surgery and radiotherapy. There is insufficient evidence in this series to say whether the results are better than those for surgery alone.

Surgical operations and their results.

Four different operations were employed : simple mastectomy ; mastectomy and dissection of axilla ; mastectomy, dissection of the axilla and removal of the pectoralis major ; and the classical radical mastectomy. Out of 599 operations the last two formed the great majority ; they were performed 108 and 456 times respectively. Simple mastectomy was only performed on the very aged, and in some cases where the carcinoma was only found on histological grounds after operation for chronic mastitis.

The operative mortality for the whole series was—

15 in 599 cases = 2·5 per cent.

4. *Symptomatology.*

The symptoms that occur between the onset of the disease and the start of treatment can be divided into two types, the primary symptom and the secondary symptom. The primary symptom is that which first draws the patient's attention to the breast ; the secondary symptom is that which occurs between the primary symptoms and treatment. In this series there were 787 cases in which the symptoms were recorded and they occurred with the following frequency :

	Percentage frequency.
Initial symptom.	
Lump noticed by patient	83·5
Pain	10
Discharge from nipple	3
Nipple retraction	2
Lump noticed by doctor	1
Ulceration	0·5
Secondary symptom.	
Pain	16
Discharge from nipple	3·5
Nipple retraction	1

It will be seen that pain was the initial symptom in one out of ten cases and was an initial or secondary symptom in one out of every four. This is higher than would be expected from the usual teaching, and it would seem that pain was a common finding in carcinoma of the breast. Its nature varied from a feeling of discomfort to a definite sharp pain that might be referred down the arm. It was not necessarily associated with glands in the axilla. It was difficult to establish any connection between the degree of pain and the state of the growth. Quite severe pain was found in some early cases, whereas, as is well known, large tumours were often completely painless. The commonest type of pain was the " niggling " or " nagging " feeling in the breast, intermittent in type.

Nipple retraction was a symptom in only 3 per cent of cases, but it was commonly found as a clinical sign. It would seem that women did not notice the change in the nipple readily.

The symptom/treatment interval.

The average delay between the onset of symptoms and reporting for treatment in this series was 10 months (7718 months in 777 cases). As would be expected, this interval was increased with the stages of the disease. Thus Stage I cases had an average delay of 7 months, Stage II cases of 10 months and Stage III cases of 18 months.

One can therefore reason that an average case has between 7 and 10 months' grace before the catastrophe of axillary gland involvement takes place. Unfortunately there is too much variance in individual growths for this figure to be of any real value. The one practical point is the tragic length of delay before seeking aid after the patient has noticed symptoms.

5. *Recurrences: Their Distribution and Relation to the Site of the Primary Growth.*

Information as to the site of recurrences was not always strictly accurate, as a general practitioner might well mention only the important recurrence, and leave out less striking or lethal sites. The following table shows the incidence and distribution of 647 recurrences:

Area of recurrence.	Number.		Percentage.
Skin:			
Breast area	138		
Multiple	4	142	22
Glandular:			
Supraclavicular	91		
Axillary	39		
Unspecified	3	133	20·5
Bone:			
Vertebra	84		
Pelvis	23		
Leg	9		
Sternum	9		
Arm	2	132	20·5
Skull	2		
Others	3		
Intrathoracic:			
Lung	123		
Mediastinum	6	129	20
Abdomen	51		8
Breast:			
Homolateral	20		
Contralateral	15	35	5·5
Brain	10		1·5
Multiple	15		2

Recurrences that are recorded under the heading of " Breast, homolateral " are those in which there has been a return of activity after treatment by radiotherapy. They should probably be called " insufficiently treated," but have been included in this analysis for convenience. Those under the heading " Breast, contralateral " are those in which the evidence strongly suggests a true recurrence rather than a second primary.

The low incidence of mediastinal recurrences is probably due to the fact that practitioners were likely to call any intrathoracic recurrences " pulmonary metastasis." These figures, however, do not lend support to Rowlands and Turner (1936), who state that the mediastinum is the commonest site for a recurrence.

This investigation has cleared up one point that has been debated in the past. There is a direct path of spread from the breast to the supraclavicular glands. Of 91 cases that developed recurrences in those glands 17 were Stage I cases, treated by radical mastectomy, and with axillary glands proved to be free from growth histologically. In view of the fact that the axillary glands were removed at operation and that path of spread permanently closed, the subsequent invasion of the supraclavicular glands must have been through a more direct path.

Relation of site of primary growth to site of recurrence.

Some quadrants of the breast have been considered to be more likely to metastasize in certain areas. Thus, inner quadrant growths have been held to give secondaries in the chest more frequently. The percentage of pulmonary and abdominal recurrences of all cases in each quadrant has been determined. The results are as follows :

Quadrant.	Percentage giving pulmonary metastasis.	Percentage giving abdominal metastasis.
Upper and outer	15	8·5
Inner and upper	14·5	6·5
Central	19	6·5
Upper and inner	20	4
Inner and lower	16	7·5
Axillary tail	21·5	7·5

There is no evidence in these figures to suggest that any quadrant of the breast has an especial tendency to metastasize to the chest or abdomen.

The interval between recurrence and death.

When attempting to give a prognosis in a case of carcinoma of the breast that has developed secondaries, some guidance may be given by the average interval in this series. It has been found to be close to 12 months for all stages of growth. The actual figures are :

Stage I	11·5 months.
Stage II	10·3 ,,
Stage III	12·5 ,,

It is of some interest to notice that delay in the onset of a recurrence does not mean that that recurrence will be of less virulence. Recurrences in Stage I

growths occurred much later than in the other stages but, once they were found, the prognosis was equally bad.

This finding is in accordance with the clinical fact that cases in which the recurrence has been delayed five or ten years are just as serious.

6. *The Ten to Fifteen Year Period.*

No surgeon who has followed cases of carcinoma of the breast can have failed to see cases of recurrence occurring at long intervals after operation. These are often regarded as interesting rarities. As a result of the long term follow-up of the cases in this series it has been found that delayed recurrence is not rare.

The liability of recurrence in each five-year period after operation can be determined by calculating the percentage of cases that develop a recurrence out of the total of cases that were alive at the beginning of the period. This gives the following interesting figures :

0–5	years	.	394	recurrences in	734	cases	.	53·5 per cent.
5–10	,,	.	50	,, ,,	199	,,	.	25 ,,
10–15	,,	.	11	,, ,,	89	,,	.	12·5 ,,

This table shows that the tendency to develop a recurrence is less with each five-year period, but is still very considerable after ten years. The actual number that do develop these late recurrences is small owing to the cases that die early, and this has given the false impression that survival to five years means that the chance of recurrence is negligible. Doubt has long been cast on the term " five-year cure rate." It would seem that the " ten-year cure rate " is equally valueless. This investigation has forced one to the conclusion that, no matter how early the case or thorough the treatment, no patient is free from the possibility of recurrence until death occurs from some other cause.

SUMMARY.

1211 cases of carcinoma of the female breast have been analysed. The following results have been found and conclusions drawn :

1. 72 per cent of all traced cases died from the disease within ten years.
2. The factor of overwhelming importance in the prognosis was the state of the axillary glands.
3. Attachment to the deep tissues was of worse significance than attachment to the skin.
4. Neither the situation of the primary nor the age of the patient appeared to affect the prognosis to any marked degree.
5. Up to 1935 ancillary radiotherapy to surgery gave worse results than surgery alone. After 1936 changes in radiotherapeutic technique gave better results than in the previous decade. There is insufficient evidence to show whether the use of radiotherapy improved the results of surgery.
6. Indirect evidence is produced to show that there is a direct channel of spread between the breast and the supraclavicular glands.
7. The average length of life after the first sign of a recurrence, no matter how long delayed, was approximately one year.
8. The liability to recurrence diminishes with each 5-year period after treatment, but is still high 15 years after treatment.

It is with pleasure that I record my acknowledgment of the help that I have received during this investigation: from the Medical Committee of the Middlesex Hospital, who gave me permission to use the records; from the surgical staff, past and present, whose cases I have used; from the staff of the Registry and Follow-up Department in helping to trace the cases; and particularly from Mr. R. Vaughan Hudson and Prof. B. W. Windeyer, who have given me their interest, helpful guidance and wise criticism.

REFERENCES.

KEYNES, G.—(1932) *Brit. J. Surg.*, **19**, 415.
PERRY, A.—(1925) *Ibid.*, **13**, 39.
'Rep. publ. Hlth med. Subj. Lond.,' No. 34, 1926.
ROWLANDS, R. P., AND TURNER, P.—(1936) 'Operations of Surgery.' London (Churchill). 8th ed., p. 825.
SAMPSON HANDLEY, W.—(1922) 'Cancer of the Breast.' London (Murray).
SCARFF, R. W., AND HANDLEY, R. S.—(1938) *Lancet*, ii, 582.

APPENDIX.

TABLE I.—*Effect of Clinical State of Growth at Start of Treatment. Stage I: All Treatments.* Total, 250.

Results	5 years. Number.	5 years. Per cent.	10 years. Number.	10 years. Per cent.
Alive	115	46·0	51	20·4
Dead: Due to operation	7	2·8	7	2·8
Dead: ,, carc. mammae	75	30·0	96	38·4
Dead: ,, unknown cause	19	7·6	28	11·2
Dead: ,, other cause	8	3·2	9	3·6
Alive with recurrence	0	0·0	3	1·2
Untraced	26	10·4	56	22·4

TABLE II.—*Stage II: All Treatments.* Total, 484.

Results.	5 years. Number.	5 years. Per cent.	10 years. Number.	10 years. Per cent.
Alive	84	17·3	38	7·8
Dead: Due to operation	11	2·3	11	2·3
Dead: ,, carc. mammae	301	62·2	330	68·2
Dead: ,, unknown cause	23	4·8	30	6·2
Dead: ,, other cause	30	6·6	35	7·2
Alive with recurrence	0	0·0	0	0·0
Untraced	33	6·8	40	8·3

TABLE III.—*Stage IIIa : All Treatments.* Total, 24.

Results.	5 years. Number.	5 years. Per cent.	10 years. Number.	10 years. Per cent.
Alive	2	8·3	2	8·3
Dead — Due to operation	0	0·0	0	0·0
Dead — ,, carc. mammae	17	70·8	17	70·8
Dead — ,, unknown cause	3	12·5	3	12·5
Dead — ,, other cause	1	4·2	1	4·2
Alive with recurrence	0	0·0	0	0·0
Untraced	1	4·2	1	4·2

TABLE IV.—*Stage IIIb : All Treatments.* Total, 78.

Results.	5 years. Number.	5 years. Per cent.	10 years. Number.	10 years. Per cent.
Alive	6	7·7	0	0·0
Dead — Due to operation	2	2·8	2	2·8
Dead — ,, carc. mammae	65	83·0	70	90·3
Dead — ,, unknown cause	0	0·0	1	1·4
Dead — ,, other cause	3	3·7	3	3·7
Alive with recurrence	0	0·0	0	0·0
Untraced	2	2·8	2	2·8

TABLE V.—*Stage I : No Attachment and Slight Attachment.*

Results.	No attachment. 5 years.	No attachment. 10 years.	Slight attachment. 5 years.	Slight attachment. 10 years.
Alive	65	29	54	24
Dead — Due to operation	3	3	4	4
Dead — ,, carc. mammae	31	44	40	52
Dead — ,, unknown cause	9	12	9	15
Dead — ,, other cause	3	3	6	7
Alive with recurrence	0	0	0	0
Untraced	14	34	12	23

TABLE VI.—*Stage I : Early Attachment, Superficial and Deep.*

Results.	Deep. 5 years.	Deep. 10 years.	Skin. 5 years.	Skin. 10 years.
Alive	20	11	33	13
Dead — From operation	1	1	3	3
Dead — ,, carc. mammae	21	24	19	28
Dead — ,, unknown cause	4	4	9	12
Dead — ,, other cause	1	3	1	2
Alive with recurrence	0	0	0	0
Untraced	5	9	8	15

It is with pleasure that I record my acknowledgment of the help that I have received during this investigation: from the Medical Committee of the Middlesex Hospital, who gave me permission to use the records; from the surgical staff, past and present, whose cases I have used; from the staff of the Registry and Follow-up Department in helping to trace the cases; and particularly from Mr. R. Vaughan Hudson and Prof. B. W. Windeyer, who have given me their interest, helpful guidance and wise criticism.

REFERENCES.

KEYNES, G.—(1932) *Brit. J. Surg.*, **19**, 415.
PERRY, A.—(1925) *Ibid.*, **13**, 39.
'Rep. publ. Hlth med. Subj. Lond.,' No. 34, 1926.
ROWLANDS, R. P., AND TURNER, P.—(1936) 'Operations of Surgery.' London (Churchill). 8th ed., p. 825.
SAMPSON HANDLEY, W.—(1922) 'Cancer of the Breast.' London (Murray).
SCARFF, R. W., AND HANDLEY, R. S.—(1938) *Lancet*, ii, 582.

APPENDIX.

TABLE I.—*Effect of Clinical State of Growth at Start of Treatment. Stage I: All Treatments.* Total, 250.

Results	5 years. Number.	5 years. Per cent.	10 years. Number.	10 years. Per cent.
Alive	115	46·0	51	20·4
Dead — Due to operation	7	2·8	7	2·8
Dead — ,, carc. mammae	75	30·0	96	38·4
Dead — ,, unknown cause	19	7·6	28	11·2
Dead — ,, other cause	8	3·2	9	3·6
Alive with recurrence	0	0·0	3	1·2
Untraced	26	10·4	56	22·4

TABLE II.—*Stage II: All Treatments.* Total, 484.

Results.	5 years. Number.	5 years. Per cent.	10 years. Number.	10 years. Per cent.
Alive	84	17·3	38	7·8
Dead — Due to operation	11	2·3	11	2·3
Dead — ,, carc. mammae	301	62·2	330	68·2
Dead — ,, unknown cause	23	4·8	30	6·2
Dead — ,, other cause	30	6·6	35	7·2
Alive with recurrence	0	0·0	0	0·0
Untraced	33	6·8	40	8·3

TABLE III.—*Stage IIIa : All Treatments.* Total, 24.

Results.	5 years.		10 years.	
	Number.	Per cent.	Number.	Per cent.
Alive	2	8·3	2	8·3
Dead — Due to operation	0	0·0	0	0·0
Dead — ,, carc. mammae	17	70·8	17	70·8
Dead — ,, unknown cause	3	12·5	3	12·5
Dead — ,, other cause	1	4·2	1	4·2
Alive with recurrence	0	0·0	0	0·0
Untraced	1	4·2	1	4·2

TABLE IV.—*Stage IIIb : All Treatments.* Total, 78.

Results.	5 years.		10 years.	
	Number.	Per cent.	Number.	Per cent.
Alive	6	7·7	0	0·0
Dead — Due to operation	2	2·8	2	2·8
Dead — ,, carc. mammae	65	83·0	70	90·3
Dead — ,, unknown cause	0	0·0	1	1·4
Dead — ,, other cause	3	3·7	3	3·7
Alive with recurrence	0	0·0	0	0·0
Untraced	2	2·8	2	2·8

TABLE V.—*Stage I : No Attachment and Slight Attachment.*

Results.	No attachment.		Slight attachment.	
	5 years.	10 years.	5 years.	10 years.
Alive	65	29	54	24
Dead — Due to operation	3	3	4	4
Dead — ,, carc. mammae	31	44	40	52
Dead — ,, unknown cause	9	12	9	15
Dead — ,, other cause	3	3	6	7
Alive with recurrence	0	0	0	0
Untraced	14	34	12	23

TABLE VI.—*Stage I : Early Attachment, Superficial and Deep.*

Results.	Deep.		Skin.	
	5 years.	10 years.	5 years.	10 years.
Alive	20	11	33	13
Dead — From operation	1	1	3	3
Dead — ,, carc. mammae	21	24	19	28
Dead — ,, unknown cause	4	4	9	12
Dead — ,, other cause	1	3	1	2
Alive with recurrence	0	0	0	0
Untraced	5	9	8	15

TABLE VII.—*Site of Growth. Stage I Cases.* Total, 240.

Inner and upper	48
Inner and lower	13
Outer and upper	89
Outer and lower	23
Axillary tail	7
Central	23
Mid-medial	1
Mid-inferior	7
Mid-lateral	4
Mid-superior	25

TABLE VIII.—*Site of Growth. Stage II.* Total, 450.

Inner and upper	60
Inner and lower	11
Outer and upper	184
Outer and lower	41
Axillary tail	20
Central	68
Mid-medial	3
Mid-lateral	21
Mid-inferior	11
Mid-superior	31

TABLE IX.—*Mortality Rate for Each Age Group in Each Stage.*

Age group.	Stage I.		Stage II.		Stage III.	
	5 years.	10 years.	5 years.	10 years.	5 years.	10 years.
0–39	41	57	78	87	100	100
40–49	26	50	73	80	89	95
50–59	38	62	66	77	87	90
60–69	37	49	67	72	89	96
70–79	36	43	61	67	75	83
80–89	67	67	100	100	33	33

TABLE X.—*Results According to Treatment. Stage I Growths.*

		Surgery only.	Surgery and radium.	Surgery, radium, DXR.	Surgery and DXR.	Radio-therapy only.
Alive		68	13	13	7	..
Dead	From operation	3	2	0	1	..
	,, carc. mammae	29	10	9	12	..
	,, other cause	9	1	1	4	..
	,, unknown cause	5	0	0	0	..
Untraced		15	4	1	2	..
		129	30	24	26	

TABLE XI.—*Results According to Treatment. Stage II Growths.*

		Surgery only.	Surgery and radium	Surgery, radium, DXR.	Surgery and DXR.	Radio-therapy only.
Alive		42	9	11	13	9
Dead	From operation	2	6	0	0	3
	,, carc. mammae	99	38	43	64	57
	,, other cause	6	2	2	5	8
	,, unknown cause	14	5	0	4	9
Untraced		20	7	0	1	5
		183	67	56	87	91

TABLE XII.—*Mortality Rates for Various Treatments. Stage I Growths.*

Treatment.	Percentage mortality rate. 5 years.	Percentage mortality rate. 10 years.
Surgery	28	49
Surgery and radium	46	68
Surgery, radium and DXR	39	50
Surgery and DXR	54	67
Radiotherapy	43	86

TABLE XIII.—*Mortality Rates for Various Treatments. Stage II Growths.*

Surgery	62	72
Surgery and radium	73	83
Surgery, radium and DXR	77	82
Surgery and DXR	74	82
Radiotherapy	70	73

THE EFFECT OF A DIET CONTAINING YEAST UPON THE DEVELOPMENT OF TUMOURS INDUCED BY 2-ACETYL-AMINO-FLUORENE.

F. BIELSCHOWSKY.

From the Department of Pathology and the Cancer Research Laboratories, University of Sheffield.

Received for publication March 25, 1947.

In a recent paper Harris (1946, 1947) reported " on the failure of liver extract and of dietary protein level to influence the production of liver tumours by 2-amino-fluorene and 2-acetyl-amino-fluorene." Working with Wistar rats he obtained a high incidence of malignant hepatomas after 25–30 weeks of feeding the acetyl-compound. His findings were in good agreement with the results obtained by me in rats of the same ancestry (Bielschowsky, 1944). Although liver extracts retarded tumour development slightly in Harris's experiment, an indication that dietary factors might possibly have an influence on the development of tumours induced by 2-acetyl-amino-fluorene, Giese, Clayton, Miller and Baumann (1946) expressed the opinion " that acet-amino-fluorene is a carcinogen the activity of which appears to be insensitive to diet." The purpose of this paper is to show that this conception needs modification, and that further efforts should be made to discover diets which protect against acetyl-amino-fluorene to the same degree as for instance a high casein, high riboflavin diet inhibits the action of dimethyl-amino-azo-benzene.

METHODS.

In experiments reported previously (Bielschowsky, 1944, 1946) each rat received daily 7 g. of dried powdered bread and 3 g. of skimmed milk powder, which amounts were raised to 8·4 and 3·6 g. respectively once the rat had reached a body weight of 100 g. This diet was supplemented by 0·25 ml. of cod liver oil and 2 g. of cabbage given once weekly. In the experiments described in this paper the diet was modified as follows :

In groups A, B and C the food consisted of 6·5 g. of dried powdered bread, 2 g. of skimmed milk powder and 1·5 g. of dried yeast with the same 20 per cent increase as mentioned above. The yeast was obtained from Messrs. Hopkin and Williams, who supplied the following information : " It is a distillers' yeast, composed of one race of yeast only. It is not debittered, because it is not bitter in the first instance. The desiccated yeast is active and can be killed by temperatures above 58° C." Before use the yeast was heated to 60° C. for several hours.

In experiment D the amount of skimmed milk was raised to 5 g. and the bread powder reduced to 5 g. ; in E 1 g. of milk powder was substituted by 1 g. of dried egg white, and in F the original (1944) diet was given. In all these experiments there was the same 20 per cent increase of food and the same supplements of cod liver oil and cabbage were given. 20–25 ml. water were consumed by each rat daily ; a daily dose of 4 mg. of 2-acetyl-amino-fluorene was given per os for 25 weeks to all the animals.

The rats belonged to the same strains used in earlier experiments, and they were killed as soon as the presence of a tumour could be established. The experiments were terminated at the end of the 42nd week.

RESULTS.

Table I gives the results of the three yeast experiments which were carried out between 1944 and 1946, using in group A and B Wistar and in C piebald rats. The numbers 1–5 refer always to males and numbers 6–10 to females.

TABLE I.—*Yeast Experiments.*

Number.	Duration of experiment.	Hepatomas.	Tumours in other organs.
A. Wistar rats.			
1	230 days	Large, malignant	0
2	251 ,,	,, ,,	0
3	293 ,,	Malignant	0
4	293 ,,	Multiple, small	0
5	293 ,,	0	0
6	293 ,,	0	0
7	293 ,,	0	Adenoma of lung.
8	293 ,,	0	Tumour of duct. acust. ext.
9	293 ,,	Small, early	0
10	293 ,,	0	Intraduct. papilloma breast.
B. Wistar rats.			
2	272 ,,	Large, malignant	0
1	286 ,,	,, ,,	0
4	293 ,,	0	0
5	293 ,,	0	0
7	293 ,,	Early, small	0
8	293 ,,	0	0
9	293 ,,	0	0
10	293 ,,	Early, small	0
C. Piebald rats.			
2	202 ,,	0	Tumour of duct. acust. ext.
1	263 ,,	Multiple, malignant	,, ,, ,, ,,
3	288 ,,	Early, small	0
4	293 ,,	,, ,,	Adenoma of lung.
5	293 ,,	,, ,,	0
6	293 ,,	0	0
7	293 ,,	0	0
8	293 ,,	0	0
9	293 ,,	0	0
10	293 ,,	0	0

0 = no cancer. Benign cystic cholangiomata, present in nearly all animals, are not listed. Nos. 1–5 males, 6–10 females.

Table II gives the results of an experiment (F) which has been chosen because it is typical of the response of Wistar rats receiving 2-acetyl-amino-fluorene and the original diet. The two other groups have been selected because they demonstrate the failure of an increased milk protein content (Experiment D) or a reduced biotin content (Experiment E) of the food to influence significantly the rate of development of tumours induced by acetyl-amino-fluorene (du Vigneaud, Spangler, Burk, Kensler, Sugiura and Rhoads, 1942).

TABLE II.—(*Wistar Rats.*)

Diet.	Rat number.	Duration of experiment.	Hepatomas.	Tumours in other organs.
D. High milk protein	5	192 days	+	0
	4	204 ,,	+	0
	3	232 ,,	0	Cancer of breast, papilloma gut.
	1	265 ,,	+	Adenoma of lung.
	2	265 ,,	+	0
	10	174 ,,	0	Cancer of breast.
	6	223 ,,	0	Tumour of duct. acust. ext.
	7	243 ,,	+	Cancer of breast.
	8	252 ,,	0	Multiple cancer of breast.
	9	260 ,,	0	Adenoma of lung.
E. Egg white	4	203 ,,	+	0
	3	203 ,,	+	0
	1	209 ,,	+	0
	2	209 ,,	+	Papilloma of gut.
	5	209 ,,	+	0
	10	181 ,,	0	Cancer of breast.
	8	216 ,,	+	Tumour duct. acust. ext.
	6	218 ,,	0	0
	7	267 ,,	0	Tumour duct. acust. ext.
	9	274 ,,	+	Two cancers of breast, carcinoma of lung, tumour duct. acust. ext.
F. Basic	4	232 ,,	+	Cancer of intestine.
	1	244 ,,	+	Tumour of duct. acust. ext.
	2	255 ,,	+	,, ,, ,, ,,
	5	279 ,,	+	,, ,, ,, ,,
	3	281 ,,	+	0
	6	176 ,,	0	Cancer of breast.
	8	183 ,,	0	Cancer of breast, tumour duct. acust. ext.
	7	209 ,,	0	Ditto.
	10	216 ,,	0	Retrobulbar carcinoma.
	9	245 ,,	+	Cancer of breast, tumour duct. acust. ext.

0 = no cancer; + = hepatoma. Benign cystic cholangiomata, present in nearly all animals, are not listed. Nos. 1–5 males, 6–10 females.

For controls to experiment C the reader is referred to tables of a previous paper (Bielschowsky, 1946), in which the results obtained in our strain of piebald rats after feeding the carcinogen for 25 weeks have been recorded. All Wistar rats of experiments D, E and F developed tumours before the end of the 40th week, with the exception of rat 6 (Exp. E), which died of an infection on the 218th day. As previously reported, 33 of 38 (25 females, 13 males) piebald rats which received the basic bread-milk diet developed cancer between the 21st and 36th week. Of the 5 remaining rats, which were all females, one died on the 281st day still free from tumours. In the other 4 rats cancers were found when they were killed on the 293rd day of the experiment.

The rate of survival was very much better in the yeast experiments. Two rats dying at an early date (Rats 3 and 6, experiment B) had to be omitted from the table. Of the 28 rats left, 22 were still alive at the end of the 42nd week; they seemed to be in excellent health and no tumours could be palpated; 14 of these were females and 8 males. However, neoplasms were found in 11 at the post-mortem examination. The commonest tumours were small hepatomas, having a diameter of 3–6 mm.; in one Wistar rat a small flat intraductal papilloma of the breast was discovered.

Histological examination of these early neoplasms frequently failed to give proof of their malignancy. A fairly large experience with the tumours induced by acetyl-amino-fluorene has taught, however, that all these early neoplasms were potentially malignant, and that they would have progressed in time to true cancers. 11 rats of the yeast series are listed in Table I as free from tumours. There was, however, one sign of the blastogenic action of acetyl-amino-fluorene—a few minute cystic cholangiomata. This type of benign tumour which we have never seen to progress to malignancy has been omitted from the tables, as in the earlier communications.

Table III records the body weights of the rats at the beginning of the experiments and on the day the carcinogen was withdrawn. There were no significant differences in gain of weight in any of the six experiments. There was, however, a difference in the liver weights between the animals receiving the basic and those

TABLE III.—*Average Weights of Rats (Groups of Five Animals).*

	4–7 weeks old.	First day of experiment.	175th day.
Experiment A	Males	72·8 g.	168·4 g.
	Females	52·6 g.	151·2 g.
Experiment B	Males	47·8 g.	158·0 g.*
	Females	44·6 g.	129·5 g.*
Experiment C	Males	53·6 g.	195·0 g.
	Females	57·8 g.	139·0 g.
Experiment D	Males	38·6 g.	182·2 g.
	Females	33·4 g.	143·0 g.
Experiment E	Males	56·6 g.	190·2 g.
	Females	70·0 g.	148·0 g.
Experiment F	Males	80·4 g.	185·2 g.
	Females	54·4 g.	139·8 g.

* Average of 4 animals.

fed the yeast diet. This difference was best seen when the average weights of the females of the piebald strain were compared. Only such animals were selected as were found to be free of hepàtomas. The average weight of the livers of the females of experiment C was 7·9 g., of the controls 11 g. Previously Wilson, DeEd and Cox (1941) had observed a diffuse hyperplasia of this organ after feeding acetyl-amino-fluorene. Harris (1947) found this hyperplasia in 30 per cent of his animals. It seemed of interest that yeast prevented the hypertrophy of the liver as well as the development of hepatomas.

DISCUSSION.

In all experiments reported in this paper the amount of carcinogen given was the same, the caloric intake similar and the percentage of bread varied only slightly. Any effects observed in the yeast series have, therefore, primarily to be attributed to the substitution of milk powder by dried yeast. Experiment E is in good agreement with Harris's findings that an increase in milk proteins failed to protect against the action of 2-acetyl-amino-fluorene. There exists now good evidence that a high casein-high riboflavin diet protects only against dimethyl-amino-azo-benzene. Even related compounds (Giese, Clayton, Miller and Baumann, 1946) are not inhibited to the same extent by such a diet. Further, Strong and Figge (1946), who reviewed the literature on this subject, did not succeed in inhibiting by a diet of liver supplemented by a combination of raw milk, riboflavin and xanthine the development of tumours induced by subcutaneous injection of methylcholanthrene. Harris's failure to protect against acetyl-amino-fluorene by a diet which inhibited the action of " butter yellow " has already been mentioned.

The results obtained in the yeast experiment are not impressive, but they seem significant since the same trend of delayed tumour growth was found in three experiments involving various litters of two different strains of rats. It would probably have been an advantage to use a smaller dose of the carcinogen in the experiments reported in this paper. A daily dose of 2 mg. of acetyl-amino-fluorene will still produce a high percentage of cancers.

It seems possible that the relatively low incidence of cancers observed by Cantarow, Paschkis, Stasney and Rothenberg (1946) after prolonged treatment of rats with acetyl-amino-fluorene was partly due to a supplement of brewers' yeast in the diet given.

The problem whether certain dietary factors are true anti-carcinogens, or protect only the liver against a damage the reaction to which leads to the establishment of cancers, could not be answered by workers who studied the effect of diet in rats receiving dimethyl-amino-azo-benzene. It was hoped that working with a more versatile carcinogen would give a clue to this question. At first sight it seems that in experiments A, B and C the development of all types of tumours was delayed. There was, for instance, a significant reduction in the incidence of mammary cancers in females of the Wistar strain. This finding, however, could be explained also by the assumption that a less damaged liver was better able to cope with an excess of endogenous oestrogen and so prevented the development of tumours of the breast. No tumours of the small intestine were found in experiment C—another fact which seemed to suggest a general anti-carcinogenic effect of the yeast diet. In unpublished experiments it has been

found that the incidence of this type of tumour could be reduced by various unrelated agents. Therefore the adeno-carcinomata of the small intestine were not considered suitable material for deciding this question. The third type of tumour significantly affected was the squamous keratinizing carcinoma of the head, which is specially frequent in the piebald strain. Only three such tumours were found, one of very small size in a Wistar and two larger in piebald males, This last result could best be used as an argument in favour of a general anti-carcinogenic action of yeast. It is felt, however, that it is too early to draw such a conclusion. As long as it is not known whether or not acetyl-amino-fluorene is converted in the liver into an "active metabolite," an important fact necessary for a proper interpretation is missing, and any discussion of the role of dietary factors in the pathogenesis of the amino-fluorene induced tumours is better postponed.

SUMMARY.

A diet containing 15 per cent dried yeast delayed significantly the development of cancers induced by 2-acetyl-amino-fluorene. The only neoplastic lesion which could not be prevented were minute benign cystic cholangiomata, which were found in all animals.

REFERENCES.

BIELSCHOWSKY, F.—(1944) *Brit. J. exp. Path.*, **25**, 1.—(1946) *Ibid.*, **27**, 135.
CANTAROW, A., PASCHKIS, K. E., STASNEY, J., AND ROTHENBERG, M. S.—(1946) *Cancer Res.*, **6**, 610.
GIESE, J. E., CLAYTON, C. C., MILLER, E. C., AND BAUMANN, C. A.—(1946) *Ibid.*, **6**, 679.
HARRIS, P. N.—(1946) *Ibid.*, **6**, 487.—(1947) *Ibid.*, **7**, 88.
STRONG, L. C., AND FIGGE, F. H. J.—(1946) *Ibid.*, **6**, 466.
WILSON, R. H., DE EDS, F., AND COX, A. J.—(1941) *Ibid.*, **1**, 595.
DU VIGNEAUD, V., SPANGLER, J. M., BURK, D., KENSLER, C. J., SUGIURA, K., AND RHOADS, C. P.—(1942) *Science*, **95**, 174.

PRODUCTION OF MUTATIONS IN MICE BY 1:2:5:6-DIBENZANTHRACENE.

J. G. CARR.

From Institute of Animal Genetics, Edinburgh.

Received for publication April 10, 1947.

In an experiment in which mice of lines CBA, JK and N were crossed, and the offspring injected with methylcholanthrene for many generations, Strong (1945) made the important discovery that genetic mutations arose as a result of the injection of the carcinogenic compound. This substance had previously been tested on *Drosophila melanogaster* (Auerbach, 1939), and found not to produce lethal mutations in this species. In an experiment in which 1:2:5:6-dibenzanthracene was being given to mice, and the offspring used for breeding, it was regarded as possible that mutations might appear, though because of the nature and size of the experiment it was felt that the chance of any being detected was so small as to be negligible. Actually several mutations were obtained.

METHOD.

If a mutation is produced in a gene of the sperm or ovum of a mouse, the offspring to which this gamete gives rise will be heterozygous for the mutation, and one-half of the sperm or ova it produces will carry the mutation. Since mutations are usually recessive they will only be detected by an inbreeding programme arried out for three generations after the mutation arises. This condition was partially fulfilled in the experiment to be described, which was actually set up with an entirely different aim.

Materials.—The experiment was part of a study of the effect of the dominant yellow (A^Y) gene on cancer susceptibility. Three inbred lines of mice were used, C_3H, CBA and RIII.

The first two lines are essentially wild-type mice, while the RIII is similar except for the presence of albinism. No phenotypically detectable mutations have arisen in any of these lines among thousands raised at this Institute, so frequent spontaneous mutations can be ruled out as a source of error. The A^Y gene is a dominant giving a beautiful yellow coloration to the coat of the mice carrying it, and is not known to exist in the pure (A^YA^Y) state. All yellow mice thus carry a lower allelomorph of the A series, and pure-breeding yellows are unknown. The A^Y gene in the present experiment was derived from a stock kept by Dr. Auerbach, in which it is combined with the a^t gene. This stock is inbred, though not by strict brother-sister matings. It would be impossible that all the mutations to be described could have come from this source, firstly because none has appeared in the A^Ya^t stock despite inbreeding, and secondly because if they were so introduced, they should have appeared frequently in the *early*

generations of the experiment, while the mutations appeared, as would be expected, seldom and in the F_3 or later generations. A single male introduced the A^Y gene into all three lines, and the yellow mice in the offspring were crossed again to the respective pure line, and this process carried on indefinitely. The end result will thus be mice essentially of pure-line make-up, but half of each litter will carry the A^Y gene. Thus the susceptibility of mice genetically uniform except for presence of absence of the A^Y gene can be studied.

As it was also felt desirable to study the effect of carcinogens on the A^Y gene in non-uniform genotypes, some yellow mice of the F_1 and F_2 backcross (i.e. 1/2 or 3/4 pure-line, 1/2 or 1/4 A^Y stock) were injected with carcinogen (P_1 treated generation), mated together, and the yellow offspring (F_1 treated) selected, injected with carcinogen and mated amongst themselves again. This was done for one more generation (F_2 treated), and partially for another generation (F_3 treated). A few F_4 mice were also raised. The carcinogen used was 1:2:5:6-dibenzanthracene, of which 0·1 c.c. of a saturated solution in arachis oil was injected subcutaneously at the age of 5–8 weeks. In all, 83 mice were treated. As the aim of the experiment was to study the histological type of tumour produced, litters were not kept and mated separately, but usually 2, 3, or 4 litters were mixed and allowed to breed together. This out-breeding must have seriously reduced the possibility of detecting mutations, and made it impossible to detect in which mouse the mutation arose. Detailed breeding records are thus not available, though it can be stated that all mutations appeared in the F_3 or later, and none in the F_1, which would be the case if the original yellow mouse was heterozygous for any mutation (see below).

It should be pointed out that the efficiency of this breeding arrangement for the detection of mutations was further reduced by three additional factors. Firstly, when yellow mice are bred together, all A^YA^Y mice, which comprise one quarter of the offspring, die before birth, and any mutations which were associated with these individuals are lost. This reduces the efficiency to 75 per cent of the possible. Secondly, about 20 per cent of the offspring were albinos, resulting from the albino genes of the RIII yellows coming together, and colour variations are invisible in such mice. This reduced the efficiency by a further 20 per cent, so that the detection of mutants was only 60 per cent of the possible efficiency. Thirdly, many mutants visible on an agouti background (e.g. brown) are not shown when the yellow gene is present. This further reduced the efficiency.

Mutants.—As described above, all mice born in this experiment should have been of three types : normal agouti, albino, or yellow. The following variants were found in addition, in the F_3 treated and later generations :—

1. Hydrocephalus : Two mice, both albinos, with typical hydrocephalus arose in different litters in one cage. Both died at the age of 14 days, and the parents were not traced. It is possible that they were the two mice which died of cancer resulting from the injection of the hydrocarbon shortly after the second litter was produced. Breeding tests were thus impossible, but it should be noted that hydrocephalus is a well-known genetic mutation in mice. It has never been seen in inbred mice or in the A^Y line mice at the Institute.

2. Absence of left horn of uterus : Found in one yellow mouse post-mortem. The parents were dead, and the genetic nature of this variant could not, therefore, be established. Nothing like this has ever been noted in the Institute mice.

3. Brain hernia : Found in one of seven 16-day embryos in a tumour-bearing yellow female. Nothing of this type has been noted in over 600 embryos of inbred lines examined, though again the genetic nature cannot be established.

4. " Brown " : Resembles the common recessive mutation *b*, and is inherited as a simple recessive.

5. " Pink eye " : Resembles the recessive genes *p* and *pa*, and is inherited as a simple recessive. Another appearance of a similar mutant, almost certainly independent, was lost before testing could be carried out.

6. " Recessive spotting " : Inherited as a recessive, and resembles the gene *s*.

7. Chinchilla : Resembles the recessive gene c^{Ch}, and like it is inherited as a recessive, allelomorphic with albinism (*c*). This mutation segregated twice during the breeding. It is possible from the breeding records that it arose from the same P_1 male (one of three in the same cage). If it arose from two mutated sperms it will represent two separate mutations. If the mutation arose in the earlier series of the germ-tract cells, it is possible that more than one sperm carrying the mutation was produced by this male, though the likelihood of these few out of the millions of sperm available being the ones to fertilize twice are remote. This second mutation is therefore probably independent, but this cannot be certain.

No attempt was made to breed further to obtain more mutants, though this might have been possible, as it was felt that a new and more efficient breeding method would give better results with the same labour.

Contamination.—In all mutation experiments the question of contamination, as opposed to mutation, arises. There are three possibilities in the present experiment, which, as will be shown, can be reasonably eliminated :—

(1) Mutants introduced by the inbred lines : As thousands of these have been raised at the Institute, all by strict brother-sister mating, without any mutants being found, it is extremely unlikely that these mutants could have been introduced by the inbred lines without detection. Furthermore, were this the case, some of the F_1 and F_2 of the mating with the A^Y mouse (which were the P_1 treated generation) would have been heterozygous for these genes, and the mutations would have appeared in quantity in the next generation of crossing, i.e. the F_1 treated. This was not the case.

(2) For the same reasons the mutations could not have been present in the original A^Y male, or they would have appeared in the yellow line or F_1 treated generation. Were he responsible, he would have had to be heterozygous for these 7 genes in addition to his own $A^Y a^t$ make-up, which would be rather an admirable genetic achievement, even if deliberately attempted.

(3) Contamination by stray mice : The whole experiment was carried out in a small room of the mousery, in which only this " yellow experiment " and inbred mice are maintained. Most of the rest of the cages were used for inbreeding experiments, which would detect any contamination by strays. No unexpected colours have ever occurred in the inbred mouse section, comprising nearly 500 breeding pairs. Contamination is thus unknown in a large control series. Furthermore, the " general purpose" mice in the mousery are of a much heavier type than the rather weedy inbreds, and on crossing with inbreds produce a strikingly larger mouse. None of the mutants was of this type, but all of the usual " inbred " size. Contamination by strays does not therefore appear to be a satisfactory explanation, and true genetic mutation must have occurred.

DISCUSSION.

The fact that this carcinogenic hydrocarbon, and also methylcholanthrene in the experiments of Strong, will both produce gene mutations, is obviously an important point, as it brings them into line with X-rays and ultraviolet light, both of which also produce mutations and cancer, and under certain conditions all will also show a therapeutic action on cancer. The parallel is striking, though a certain amount of caution is necessary before erecting elaborate theories on the basis of these observations. For example, it is not yet proved that the mutations are due to the carcinogen, and not to a metabolic product. In this connection the negative result on *Drosophila melancgaster* with carcinogenic hydrocarbons is perhaps significant. Furthermore, compounds such as acetylaminofluorene and urethane produce cancer, but have not yet been shown to produce mutations, while mustard gas and analogues will produce mutations, but have not yet been demonstrated to have any carcinogenic action (Auerbach, Robson and Carr, 1947), though they possess an anti-carcinogenic effect. Clearly, much work remains to be done before these results can be safely used as a basis for generalizations.

They do, however, raise one disquieting point. Cancer due to the carcinogenic hydrocarbons is a recognized industrial hazard, but it is now possible that this is not their only effect, but that persons subjected to the prolonged action of these materials may suffer germinal mutations. Though considerable discussion has been given to the question of mutations produced by atomic bomb explosions, it is possible that a much more frequent cause of these disturbances may have been unsuspectedly in action for many years on persons exposed to the influences of carcinogenic hydrocarbons. While mutations analogous to some described in this paper, e.g. reduction of hair and eye pigments, may be unimportant in humans, other types of mutants, some not detectable by the methods used, would be a serious factor in public health. Hydrocephalus, found in this work, certainly would be. Dominant lethals, leading to sterility or miscarriages, may also be produced, unimportant in mice where a large litter size is found, but of serious concern in man. It is possible that some mutants, such as the brain hernia found as an embryo, would normally be missed by the scheme used above, as mice usually eat abnormal and inviable young. The same unconcerned and easy solution of such difficulties is not the case in humans. There are possible also dominant mutations, which would appear in the first generation. Though none was found in the series above, several were reported by Strong (1945) using methylcholanthrene. It is obviously desirable that this additional hazard of exposure to these carcinogens should be evaluated.

That this need is urgent is also suggested by the following line of argument: Radiation mutations are almost entirely random, i.e. if a certain gene is mutated by an ionization in one sperm, the chance that the same gene will mutate in another sperm in the same or another individual is not increased. The efficiency of mutation with regard to any given gene is thus almost zero. But this is not necessarily the case with chemicals. If a chemical distributed via the blood stream reacts with a given locus to produce a mutation in one sperm, it is obviously liable to do the same with all other similar loci in other sperms (or ova). An efficiency of mutation at a given locus at 100 per cent can thus be imagined, and then *all* offspring of an exposed individual will carry the abnormal gene. A mating of any two exposed individuals would then give *all* F_1 of the mutated type, a state which does not arise until the F_4 of close inbreeding with radiation-

induced mutations. Even at a 5 per cent efficiency of mutation, two exposed individuals would produce 5 per cent + 5 per cent = 10 per cent with the gene in single dose, and 5 per cent × 5 per cent = 1/400 offspring with a double dose of the mutant gene.

A fairly high efficiency of this type seems likely because in the experiment above, 7 mutations from the limited number of animals bred is far above the expectation were X-rays used as a source of mutations. In fact, before this efficiency aspect was realized, it was regarded as unlikely that the experiment would yield even one mutation. Estimates suggest at least 100,000 genes as the number in a mouse, of which only about 100 may have visible effects. Only 1 in 1000 mutations can thus be detected by inspection. The others are either invisible (e.g. blood-group mutations) or otherwise lost (e.g. lethals). But as well as efficiency at certain loci, chemicals may not affect all points on the chromosome, so that only certain genes may mutate. They could therefore have a high efficiency at certain loci resulting in a high frequency of abnormal offspring without causing death of the cell, as happens when concentrated doses of irradiation are given to increase mutation rate.

It is obviously tempting to ascribe the ability of the carcinogenic hydrocarbons to produce mutations to their complex system of conjugated double bonds. This system would readily pick up energy from the surroundings, and liberate it with destructive effect at one point. The carcinogenic hydrocarbons also are well known to combine with active materials (e.g. picric acid, maleic acid) more readily than the non-carcinogenic ones, and this ability can be related to their structure. Combination with genetic material is thus likely. It is interesting to note that the other major class of chemical mutagens, the nitrogen and sulphur mustards, will similarly combine readily with gene material, and their effects suggest that the energy changes they produce in the chromosomes, which are of the chemical order, and not as great as those of high intensity irradiation, are quite sufficient for mutation. The types of mutants produced are somewhat different from those produced by high-energy radiation, and of a type which agrees with the suggestion that less energy is involved in the mutation (e.g. mosaic production) (Auerbach, Robson and Carr, 1947). The hydrocarbons are possibly even less energetic, and thus may only be able to produce mutations in genes that are of less stability than most. This would produce some degree of specificity as required above, and it is significant that most of the mutations produced both here and by Strong are already known, i.e. this suggests that genes whose unstable nature leads to spontaneous mutation are most readily attacked.

SUMMARY.

Mice were injected with 1:2:5:6-dibenzanthracene and their offspring similarly treated and mated among themselves for up to four generations. Seven variants were obtained, of which four were proved to be recessive gene mutations. As the efficiency of the breeding method for the detection of mutations was very low, this probably represents only a few of the mutants actually produced.

REFERENCES.

AUERBACH, C.—(1939) *Proc. Roy. Soc. Edin.*, **60**, 164.
Idem, ROBSON, J. M., AND CARR, J. G.—(1947) *Science*, **105**, 243.
STRONG, L. C.—(1945) *Proc. Nat. Acad. Sci.*, **31**, 290.

CARCINOGENIC CONSTITUENTS OF COAL-TAR.

I. BERENBLUM AND R. SCHOENTAL.

From the Oxford University Research Centre of the British Empire Cancer Campaign, Sir William Dunn School of Pathology, University of Oxford.

Received for publication April 22, 1947.

THE early work of Bloch and Dreifuss (1921), of Kennaway (1924*a*, 1924*b*, 1930), and others, on the chemistry of carcinogenic tars (see reviews by Woglom, 1926; Watson, 1932; Seelig and Cooper, 1933), pointed the way to the final isolation and identification of 3:4-benzpyrene (Cook, Hewett and Hieger, 1933), and thus laid the foundation of the new phase of cancer research concerned with the synthesis of carcinogenic compounds of known constitution (see reviews by Cook, Haselwood, Hewett, Hieger, Kennaway and Mayneord, 1936; Cook and Kennaway, 1938, 1940; Fieser, 1938, 1940; Hartwell, 1941; Cook, 1942).

However, with the rapid advance in the work on the synthetic carcinogens, interest in carcinogenic tars diminished, and the previous indications that coal-tar might possibly contain potent carcinogens other than benzpyrene (Hieger, 1937), were not followed up. There has since been a growing tendency in the literature to accept benzpyrene as the only carcinogen present in tar, as exemplified by the spectrographic work of Miescher, Almasy and Zehender (1941), despite the biological evidence (Schürch, 1939; Berenblum, 1945*a*; and others) of striking differences between the carcinogenic action of tar and that of benzpyrene (e.g. when tested on rabbit's skin.

The present re-investigation of carcinogenic constituents of tar, which was begun in 1941, was undertaken in order to determine whether carcinogens other than benzpyrene did, in fact, exist in tar, and if so, whether their properties were significantly different from those of the known carcinogens.

METHODS.

A horizontal retort tar of high carcinogenic potency, obtained from the City of Leeds Gas Department, was used for this investigation. The methods of fractionation included (1) extraction with benzene, light petroleum (b.p. 60–80° C.), or mixtures of the two; (2) chromatography, using alumina ("Aluminium Oxide for Adsorption Purposes," B.D.H. Ltd.) in columns ranging from 1–12 kg. capacity; (3) high vacuum distillation (at 0·05–0·2 mm. Hg); and (4) crystallization, formation of picrates, etc.

Since the object of the biological tests was to determine in which of the fractions the carcinogens were present in high concentration, rather than to evaluate the exact potencies of the weaker fractions, relatively small groups of animals were used for each test, and the experiments only continued for a sufficient time to enable us to recognize rapidly which were the most active fractions. Consequently, in the results recorded below, failure to produce tumours does not necessarily denote *complete* absence of carcinogenic activity.

Tests for carcinogenic action were carried out by skin painting; once weekly in mice and twice weekly in rabbits. In the early stages of the work, all fractions

were tested on mice as well as on rabbits ; in some of the later experiments the tests were confined to rabbits.

Throughout the course of the work samples were examined for fluorescence spectra, and these criteria, as well as chromatographic behaviour and tendency for crystallization, were used as guides for separation into fractions for biological testing. In many cases the amount of benzpyrene was estimated quantitatively. (For method, see Berenblum and Schoental, 1943). In later stages, melting points, formation of picrates, and other properties were used as criteria for separation.

EXPERIMENTAL.

As a preliminary investigation, 500 ml. of a 5 per cent solution of tar in benzene was passed through a column of alumina, the column developed with benzene, and the combined eluates (fraction *BTE*) kept for carcinogenic testing. The adsorbed material on the column was eluated with acetone (fraction *BTA*-1), and then with chloroform containing 1 per cent acetic acid (fraction *BTA*-2). The results of skin painting with these fractions are shown in Table I, and may be compared with those of a 5 per cent solution of untreated tar (*BT*).

These results show that the carcinogenic constituents of tar are concentrated in the benzene eluate from an alumina column.

A further sample of fraction *BTE* in benzene (obtained from 100 g. of tar) was diluted with two volumes of light petroleum, filtered, and the filtrate passed through a column of alumina. This was developed with a mixture of benzene and light petroleum (1 : 3), which yielded the eluate fraction *EE* ; the column was then developed with benzene alone, yielding the moderately adsorbed fraction *TE*-2 ; and then with ethanol, yielding the more strongly adsorbed fraction *TE*-1. The early (eluate) fraction *EE*, which was found to be carcinogenic to both mice and rabbits (see Table I), was re-chromatographed from light petroleum alone, yielding a filtrate fraction *EEF*, which was non-carcinogenic, and an adsorbed fraction (subsequently eluted with benzene), which was strongly carcinogenic.

Thus the carcinogenic constituents of tar were found to be adsorbed on alumina from light petroleum, and eluted from it by benzene or a mixture of benzene and light petroleum.

Since these properties were similar to those of 3:4-benzpyrene itself, a more detailed chromatography was necessary to determine whether any active fractions could be separated from benzpyrene by these means. For this the development of the column was carried out with light petroleum alone, in the first instance, then with mixtures of light petroleum and benzene, using progressively higher concentrations of the latter, and finally with benzene alone. The successive batches were examined spectrographically, and those showing similar fluorescence bands were combined, to provide the following fractions :

TS-A : containing fraction before the appearance of anthracene bands ;
TS-B : containing all fractions with anthracene bands predominating ;
TS-C : fractions after *TS-B*, but before the appearance of benzpyrene ;
TS-D : all fractions in which benzpyrene bands were detectable ;
TS-E and *TS-F* : early and late fractions, respectively, after *TS-D*.

From previous tests it was known that fractions *TS-A* and *TS-B* would be carcinogenically inactive and the present tests were, therefore, confined to the

other four fractions. These were all found to be carcinogenic both to mice and rabbits, though only one of them (*TS-D*) contained spectrographic evidence of benzpyrene.

This established the fact that there were at least two potent carcinogens in tar apart from benzpyrene, one appearing chromatographically before and the other after benzpyrene.

A further, more detailed, chromatographic separation of fraction *TS-E*, into fractions α, β and γ elicited the further information that the carcinogenic constituents which followed benzpyrene in the chromatographic column, did so fairly closely (i.e. occurred mainly in fraction α).

Of the two solvents used—benzene and light petroleum—the latter seemed the more suitable for further work, partly because of the low solubility in it of a large bulk of inactive material from tar, and partly because its use rendered careful chromatographic separation more feasible. It was first necessary, however, to devise a practical method for the exhaustive extraction of tar with light petroleum, and secondly, to determine whether all the carcinogenic constituents were taken up by this solvent. The former problem was solved by mixing the tar with about five times its volume of sand, and shaking the mixture vigorously with successive lots of light petroleum. By this means the tar remained in discreet particles (coated round the grains of sand), and thus facilitated adequate extraction. Carcinogenicity tests then showed that the carcinogenic components were confined to the petroleum—soluble fraction (compare results of fractions *PE-S* and *PE-I*, in Table I).

Using this method, 500 ml. of tar were extracted with light petroleum, and the 5 L of extract passed through a large column containing about 11 kg. of alumina. The column was developed with several litres of light petroleum, followed by mixtures of this with progressively increasing amounts of benzene, and the eluates were collected in separate lots of about 2 L each. These were examined spectrographically, and those possessing similar fluorescent spectra were combined, evaporated to a small bulk, and dissolved in small amounts of benzene, providing the following fractions for testing :

PES-A : fractions before the appearance of anthracene bands ;

PES-B : those with anthracene bands predominating ;

PES-C : those after anthracene, but before benzypyrene ;

PES-D : all fractions containing benzypyrene bands ;

PES-E : subsequent fractions, with recognizable fluorescence bands at 412 and 430mμ. ;

PES-F : later fractions with main fluorescence band at 391 mμ. ;

PES-G : still later fractions with fluorescence band at 385 mμ.

In rabbits, skin tumours were obtained with fractions *PES-C*, *PES-D*, *PES-E*, *PES-F*, and somewhat more slowly with *PES-G*, while in mice skin tumours appeared only with fractions *PES-D* and *PES-E*.

Two interesting points arise from these results : The first is the confirmation of the previous experiments, mentioned above, that fractions appearing *before* and *after* the benzpyrene-containing fraction, are highly carcinogenic. The second is that the one appearing before benzpyrene, is far more potent for the rabbit's than for the mouse's skin.

In a second fractionation, starting with larger quantities of tar (4 L), the same procedure was adopted, except that the original petroleum extract was shaken

TABLE I.

Series.	Procedure and material.	Mice. Duration of experiment.	Mice. Time of 1st tumour (weeks).	Mice. Animals with tumours. (a)	Rabbits. Duration of experiment.	Rabbits. Time 1st tumour (weeks).	Rabbits. Animals with tumours. (a)
BT	5 per cent tar in benzene (filtered)	18 weeks	11	8/20 (16)	14 weeks	7	6/7 (7)
BTE	Chromatography of BT : benzene eluate	„	8	14/20 (14)	„	7½	6/6 (6)
BTA–1	„ „ : 1st adsorbate (eluted with acetone)	„	—	0/20 (17)	„	—	0/7 (7)
BTA–2	„ „ : 2nd adsorbate (eluted with chloroform and acetic acid)	„	—	0/10 (9)	„	—	0/6 (6)
EE	Chromat. of BTE from benz. and petr.: weakly adsorbed fraction	14 weeks	10	6/10 (9)	11 weeks	7½	2/5 (5)
TE–2	„ „ „ „ „ : moderately adsorbed fraction	„	9	5/10 (10)	„	9½	2/5 (5)
TE–1	„ „ „ „ „ : strongly absorbed fraction	„	12	1/10 (10)	„	—	0/5 (5)
EEF	Rechromat. of EE from petr. alone : filtrate fraction	„	—	0/10 (10)	„	—	0/5 (5)
EEA	„ „ „ „ : adsorbate fraction	„	7	5/10 (5)	„	6½	5/5 (5)
TS–C	Chromat. of tar from petr.-benz. : fract. after anthracene but before benzpyrene	16 weeks	12	3/10 (10)	15 weeks	10	2/5 (2)
TS–D	„ „ „ „ : benzpyrene containing fraction	„	11	5/10 (10)	„	12½	2/5 (3)
TS–E	„ „ „ „ : fraction 1 after benzpyrene	„	13	6/10 (8)	„	8½	4/5 (4)
TS–F	„ „ „ „ : fraction 2 after benzpyrene	„	10	4/10 (7)	„	15	1/5 (2)
α	Rechromat. of fraction TS–E : early fraction	„	7	7/10 (8)	„	10	5/6 (6)
β	„ „ „ „ : intermediate fraction	„	9	2/10 (7)	„	8	3/6 (6)
γ	„ „ „ „ : late fraction	„	—	0/10 (3)	„	9	2/6 (6)
PE–S	Extraction of tar with light petroleum : soluble fraction	15 weeks	10	5/10 (10)	14 weeks	7	5/5 (5)
PE–1	„ „ „ „ „ : insoluble fraction (Both tested in benzene.)	„	—	0/10 (7)	„	—	0/5 (5)
PES–C	Chromat. of PE–S : fraction after anthracene, before benzpyrene	16 weeks	—	0/20 (10)	16 weeks	8½	4/5 (5)
PES–D	„ „ : fraction containing benzpyrene	„	8	10/20 (14)	„	7½	3/5 (5)
PES–E	„ „ : next fraction with fluor. bands at 412 and 430 mμ.	„	7	5/20 (10)	„	8	3/5 (5)
PES–F	„ „ : next fraction with fluor. bands at 391 mμ.	„	—	0/20 (9)	„	7½	3/5 (5)
PES–G	„ „ : next fraction with fluor bands. at 385 mμ.	„	—	0/20 (14)	„	11½	2/5 (5)

II	Chromat. of PE–S (acid and alkali treated) : fr. after after anthracene, but before benzpyrene	28 weeks	—	0/11 (10)	17 weeks	$6\frac{1}{2}$	5/5 (5)
III	" " " " " : benzpryene cont. fraction	20 "	10	7/10 (8)	"	$8\frac{1}{2}$	5/5 (5)
IV	" " " " " : fraction after benzpyrene	28 "	10	3/10 (3)	"	10	4/5 (4)
V	" " " " " : strongly adsorbed residue	19 "	—	0/10 (1)	"	—	0/5 (3)
III cryst	Crystalline fractions of III	27 "	19	2/10 (6)	17 weeks	—	0/5 (5)
IV "	" " IV	27 "	—	0/10 (8)	"	—	0/5 (5)
Fr. 3	Vacuum distillation of tar : 120–140 C. at 0·1 mm. Hg.	30 weeks	—	0/12 (7)	20 weeks	—	0/5 (4)
4	" " " : 140–160 C. "	28 "	20	1/11 (2)	"	$4\frac{1}{2}$	2/5 (4)
5	" " " : 160–180 C. "	30 "	23	4/10 (8)	"	$5\frac{1}{2}$	5/5 (5)
9a	" " " : 180–200 C. "	30 "	11	6/20 (4)	"	11	5/5 (5)
PA	" " " : 145–160 C. "	..	..	..	18 weeks	$13\frac{1}{2}$	2/6 (6)
PB	" " " : 160–170 C. "	..	..	..	"	10	3/6 (6)
PC	" " " : 170–180 C. "	..	..	..	"	9	5/6 (6)
PF	Chromat. of PB and PC (combined) : an intermediate fr. (before benzpyrene)	..	..	..	"	7	5/6 (6)
PH	" " " " : later (main) fr. (incl. benzpyrene)	..	..	..	"	7	4/6 (6)
PI	" " " " : later fr. still (after benzpyrene)	..	..	..	"	11	3/6 (6)
PD	Mixtures of crystalline fractions and decomposed picrates obtained from fractions PF and PH	..	..	..	"	—	0/6 (6)
PE		..	..	..	"	—	0/6 (6)
PG		..	..	..	"	—	0/6 (6)
PJ		..	..	..	"	—	0/6 (6)

(*a*) The figures in these columns represent animals with tumours/total number of animals used. Figures in brackets denote the number of surviving animals at the end of the experiment.

with several lots of dil. HCl (to remove basic constituents) and then with dil. NaOH (to remove phenolic and other acidic constituents), and separated into the following fractions for testing :

I : corresponding approximately to the previous fractions *PES-A* and *PES-B* ;
II : corresponding approximately to the previous fractions *PES-C* ;
III : ,, ,, ,, ,, ,, *PES-D* ;
IV : ,, ,, ,, ,, ,, *PES-E* and *PES-F* ;
V : ,, ,, ,, ,, ,, *PES-G* and residue.

The results of carcinogenic tests were essentially the same, namely, that fractions III and IV were active to both mouse and rabbit skin, whereas the earlier fraction II was highly active to rabbit skin, but not to mouse skin.

When these fractions were concentrated and allowed to stand, crystalline constituents came out of solution. These were tested separately, and while neither those of fraction III, nor of fraction IV were active to rabbit skin, the former were slightly active to mouse skin. (The low activity suggested, however, that contamination with the mother liquor may have been responsible for the few tumours which did arise.)

Further attempts were made to isolate the carcinogenic constituents from fraction II by crystallization, formation of picrates, and fractional distillation, but in each case the activity remained in the mother liquor or residue. In the end the amount of material left was too small to proceed with further fractionation.

Starting once again from whole tar, a different method of fractionation was attempted, namely, by high vacuum distillation, in the first instance, followed by chromatography, etc. :

A litre of tar was fractionally distilled *in vacuo*, at first under about 30 mm. Hg and then under 0·1 mm. Hg, yielding the following fractions :

	Temp. range.	Vacuum.	Yield.	Estimated benzpyrene content.
1	— 140° C.	at 30 mm. Hg	250 g.	—
2	80–120° C.	,, 0·1 mm. Hg	200 g.	—
3	120–140° C.	,, ,,	50 g.	—
4	140–160° C.	,, ,,	65 g.	—
5	160–180° C.	,, ,,	80 g.	Less than 0·05 g.
6	180–225° C.	,, ,,	90 g.	0·80 g.
7	225–260° C.	,, ,,	75 g.	2·00 g.
8	Residue (pitch)		250 g.	More than 0·70 g.

Note : Since the extraction of the pitch residue was inadequate, and since the estimation of this fraction was rendered difficult because of much diffuse fluorescence, the figure of 0·70 g. of benzpyrene is probably very much on the low side.

Fractions 6 and 7 were then recombined, and refractionated, yielding :

		Yield.	Estimated benzpyrene content.	
9*a*	180–200° C. at 0·1 mm. Hg	39 g.	0·24 g.	
9*b*	200–220° C. ,, ,,	63 g.	1·10 g.	2·40 g.
9*c*	220–240° C. ,, ,,	50 g.	1·30 g.	
9*d*	Residue	12 g.	0·03 g.	

Of these, fractions 3, 4, 5 and 9*a* were tested on mice and rabbits for carcinogenic action. Fraction 3 proved to be inactive, while of the other three, fraction 5 was the most active for the rabbit, and fraction 9*a* was the most active for the mouse skin.

In a second fractionation of a similar kind, using 5 L of tar, and tested on rabbit skin only, a fraction coming over at 145–160° C. (at 0·1 mm. Hg) was moderately carcinogenic; the next fraction coming over at 160–170° C. was somewhat more active, while the highest activity was obtained with the next fraction, coming over at 170–180° C.

The combined fraction of 160–180° C. was then chromatographed from light petroleum, and collected into four fractions:

(*a*) A very early fraction, containing naphthalene and most of the anthracene.

(*b*) An intermediate fraction, containing in addition to the remainder of the anthracene, fluoranthene, a large amount of chrysene, and probably chrysene homologues (see below).

(*c*) A large third fraction, containing all the benzyprene, also some chrysene, etc.

(*d*) A late fraction, in which 1:2-benzcarbazole was recognized.

These four fractions were tested on rabbit skin; fractions (*b*) and (*c*) (referred to as *PF* and *PH* in Table I) was found to be highly carcinogenic, while (*d*) (fraction *PI*) was less so.

These fractions, on concentration, deposited crystalline material, which was collected and recrystallized several times from different solvents. One of the components proved to be chrysene, crystallizing in the first crop. The second crop of colourless scales, melting at about 175–194° C., and more soluble in light petroleum than chrysene, gave a mixture of complexes with 2:7-dinitroanthraquinone of deep crimson colour (m.p. 250–275° C.), may well have contained a mixture of isomeric homologues of chrysene, difficult to separate. These crystalline fractions, as well as some others not identified, proved to be biologically inactive.

In fraction (*b*), fluoranthene was identified (m.p. 113–115° C., not depressed by mixing with an authentic specimen). This was also non-carcinogenic to the rabbit skin.

The supernatents from the crystalline fractions were further fractionated by the formation of picrates. Though many crystallizable picrates were obtained, the substances isolated by their decomposition and crystallization presented great difficulties in their final purification (as judged by sharp m.p.), and were mostly tested as mixtures (fractions *PD*, *PE*, *PG*, and *PJ*). None of them produced tumours.

CONCLUSIONS.

The results described above make it abundantly clear that coal-tar contains potent carcinogens apart from 3:4-benzpyrene. These carcinogenic constituents are soluble not only in benzene (in which the bulk of the tar constituents are readily soluble), but also in light petroleum (in which many of the tar constituents are insoluble). They are neither acids nor bases, since preliminary treatment with alkali and acid, respectively, does not remove them. By fractional distillation, these carcinogens appear slightly earlier than 3:4-benzpyrene. By chromatography on alumina, they appear fairly closely associated with benz-

pyrene, but can be separated from the latter by careful development of the column, yielding a fraction, eluted before benzpyrene, which is much more potent on rabbit than on mouse skin, and a fraction, eluted after benzpyrene, which is carcinogenically active to both mouse and rabbit skin.

Of these two major fractions the chromatographically early fraction is of particular interest, since it is probably responsible for the striking difference in carcinogenic potency between tar and 3:4-benzpyrene, when tested on rabbit skin. In this respect it also differs from the action of 9:10-dimethyl-1:2-benzanthracene (Berenblum, 1945), as indicated in Table II.

TABLE II.

	Carcinogenic potency.*	
	Mouse skin.	Rabbit skin.
Tar	VIII	X
Early fraction of tar	? 0 (<4)	IX
3:4-benzpyrene	VIII	V
9:10-dimethyl-1:2-benzanthracene	X	XI

* Grading according to the method of Berenblum (1945*b*).

From the chromotographic and other data presented in this communication it seems probable that the carcinogen appearing before benzpyrene (and highly active on rabbit skin) belongs to the class of polycyclic hydrocarbons of the 4, 5, and 6 ring systems and their homologues, and is perhaps more likely to be a homologue of a 4 ring hydrocarbon.

The present information may serve as a guide as to the preliminary procedure required for the ultimate isolation of the active carcinogenic constituents; this includes:

1. Fractional distillation at 0·1 mm. Hg, using the fraction coming over between 160–180° C.

2. Chromatography of this fraction on alumina from light petroleum; development of the column with light petroleum containing progressively increasing amounts of benzene; and using the fraction which is eluted just before benzpyrene.

3. Concentration of this fraction, and discarding the early crystals (which are inactive); and—

4. Formation of picrates and discarding the early, well-crystallizable ones.

The final mother-liquor, containing the active fraction, represents more than a two-hundred-fold concentration of the original tar.

SUMMARY.

A carcinogenic tar was fractionated by (1) extraction with solvents, (2) chromatography on alumina columns, (3) high vacuum distillation, and (4) crystallization, formation of picrates, etc.

The fractions were tested for carcinogenic activity, by skin painting in mice and rabbits, and for benzpyrene content, by fluorescence spectography.

Several of the fractions that were entirely free from 3:4-benzpyrene, were found to be carcinogenic. One of these, which appeared just before benzpyrene on the chromatography column, possessed the unusual property of high carcinogenic potency for rabbit skin and none for mouse skin.

The final active material obtained by the above methods of fractionation, represented more than a two hundred-fold concentration of the original tar. It still consisted, however, of a mixture of substances, and the active constituent has not yet been identified.

We are indebted to the City of Leeds Gas Department for a generous supply of Horizontal Retort Tar. We wish to thank Mr. H. W. Wheal for valuable technical assistance.

REFERENCES.

BERENBLUM, I.—(1945*a*) *Cancer Res.*, **5**, 265.—(1945*b*) *Ibid.*, **5**, 561.
Idem AND SCHOENTAL, R.—(1943) *Brit. J. exp. Path.*, **24**, 232.
BLOCH, B., AND DREIFUSS, W.—(1921) *Schweiz. med. Wschr.*, **51**, 1033.
COOK, J. W.—(1942) *Ann. Rep., chem. Soc.*, **39**, 155–191.
Idem, HASELWOOD, G. A. D., HEWETT, C. L., HIEGER, I., KENNAWAY, E. L., AND MAYNEORD, W. V.—(1936) *2nd Internat. Cancer Congr., Brussels*, **1**.
Idem, HEWETT, C. L., AND HIEGER, I.—(1933) *J. chem. Soc.*, 395.
Idem AND KENNAWAY, E. L.—(1938) *Amer. J. Cancer*, **33**, 50.—(1940) *Ibid.*, **39**, 381, 521.
FIESER, L. F.—(1938) *Ibid.*, **34**, 37.—(1940) *Univ. Pennsylvania Bicent. Conf.*
HARTWELL, J. L.—(1941) Washington D.C. Federal Security Agency, U.S. Publ. Health Service.
HIEGER, I.—(1937) *Amer. J. Cancer*, **29**, 705.
KENNAWAY, E. L.—(1924*a*) *J. Indust. Hyg.*, **5**, 462.—(1924*b*) *Brit. med. J.*, **1**, 564.—(1930) *Biochem. J.*, **24**, 497.
MIESCHER, G., ALMASY, F., AND ZEHENDER, F.—(1941) *Schweiz. med. Wschr.*, **71**, 1002.
SCHÜRCH, O.—(1939) *Z. Krebsforsch.*, **49**, 353.
SEELIG, M. G., AND COOPER, Z. K.—(1933) *Amer. J. Cancer*, **17**, 589.
WATSON, A. F.—(1932) *Cancer Rev.*, **7**, 445.
WOGLOM, W. H.—(1926) *Arch. Path.*, **2**, 533, 709.

ON THE BEARING OF TIME ON THE NEOPLASTIC ACTION OF SMALL QUANTITIES OF α-OESTRADIOL IN THE ENDOMETRIUM OF GUINEA-PIGS.*

A. RIESCO.

From the Department of Experimental Medicine of the National Health Service, Santiago de Chile.

Received for publication April 22, 1947.

It has been shown by Lipschutz and his associates that the tumorigenic action of oestrogens depends upon its *continuous* absorption (Lipschutz, 1942; see also Iglesias, 1938). The following two facts may be mentioned here. The quantity of α-oestradiol necessary to maintain the uterine weight of a castrated guinea-pig at the normal level of about 1 g. is somewhat less than 1 μg. given three times weekly by subcutaneous injections (Lipschutz, Rodríguez and y Vargas, 1939). On the other hand the quantity necessary to induce abdominal fibroids is not much above the mentioned hysterotrophic dose; abdominal fibroids can be induced by three injections per week of 5 μg. of α-oestradiol, or even less, when administered as the 17-caprylic ester of α-oestradiol, which is absorbed so slowly that a continuous oestrogenic action is made possible (Lipschutz, Bellolio, Chaume and Vargas, 1941). Similarly a few micrograms of free α-oestradiol or oestrone absorbed from a subcutaneously implanted tablet also are sufficient to induce fibroids (Lipschutz, Thibaut and Vargas, 1942). On the contrary, quantities about a hundred times greater are necessary to elicit the same tumorigenic abdominal reaction if administered by subcutaneous injections of the free hormone (Lipschutz *et al.*, 1939, 1942).

In the present work we shall deal with abdominal fibroids induced by extremely small quantities of estrogens and with the neoplastic action of these quantities on the uterine glands in experiments of long duration.

Fibromatogenic Action of Very Small Quantities of α-oestradiol and of Oestradiol-dipropionate.

Tablets consisting of one part of the oestrogen and 19 parts of cholesterol Merck were prepared in the usual way by mixing, dissolving in ether, drying and mixing again. Tablets weighed 33 to 35 mg. each. After drying *in vacuo* they were implanted beneath the skin into castrated female guinea-pigs weighing 380 to 480 g. Necropsy was made 112 to 118 days later. Uterine and other abdominal fibroids were elicited under these experimental conditions (Riesco, 1942), though the incidence was less than with the injection of great quantities of oestradiol administered as the benzoic ester, as in the experiments of Iglesias (1938).

Absorption was calculated from the loss of weight of the tablets recovered during necropsy, cleaned and dried subsequently. Calculation was made under

* This work has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research, grant administered by Prof. A. Lipschutz.

the supposition that the concentration of oestrogen in the tablet has not changed, as has been shown in this Department by Fuenzalida (1944) for different steroids. Such a calculation is a very rough one, since it is known that absorption is at the beginning greater than later on. In our work, with only 5 per cent of oestrogen in the tablet, chemical control of concentration was impossible. A biological control of the recovered tablets was made by reimplanting tablets a second, third and fourth time in different animals (Riesco, 1944). The result of these experiments is given in Table I.

As seen from Table I, tablets containing originally 5 per cent of the free α-oestradiol and remaining for about 4 months in the body of a guinea-pig produce anew oestrogenic and fibromatogenic actions when reimplanted into another guinea-pig. The same result was observed when 4 months afterwards the tablet was implanted for a third time and a fourth time. There was in all these experiments opening of the vagina, growth of the nipples and uterine bleeding. At necropsy the uterus was greatly increased and often monstrous, weighing 17 and even 34 gr., in animals with tablets which before implantation were already active in other animals for almost a year (see tablet No. 9 and No. 10, fourth implantation). There were uterine and other abdominal fibroids in most of these animals. Fibromatogenic action did not diminish with tablets which had been already used in previous implantations.

The total quantity of oestrogen in these tablets weighing 33 to 35 mg. was 1650 to 1750 μg. Should there have been selective absorption, oestrogenic hysterotrophic and fibromatogenic actions or uterine bleeding would have been impossible, in any case, in the third and fourth implantation. When roughly calculating the average daily absorption from the loss of weight of the tablet and supposing non-selective absorption, figures ranging between 0·9 and 1·5 μg. were obtained for the first 4 months. It is remarkable that in successive implantation smaller figures were obtained. Allowance must be made for entering of substances into the tablet as has been shown by different authors (Bishop and Folley, 1944; Deanesly and Parkes, 1943). Absorption was most probably greater than indicated by figures in our Table I. But it can easily be demonstrated that daily absorption of oestradiol was *certainly less than* about *3 to 4 μg.* In these experiments the total quantity of oestradiol in the tablet was never more than 1750 μg., and the tablet remained beneath the skin during 470 days without exhaustion of the oestrogen. But in the next paragraph we shall mention experiments in which similar tablets were active for a total of *675 days without the oestrogen being exhausted.*

Histological examination of the uterus in the mentioned animals revealed pronounced cystic glandular hyperplasia of the endometrium, polypous proliferation of the latter, thickening of the vaginal wall and proliferation of mammary tissue. These statements coincide with what has been formerly observed with the prolonged action of estrogens in this Department.

Neoplastic Action of Small Quantities of Oestrogens on the Uterine Glands in Experiments of Long Duration.

In the above-mentioned preliminary experiments evidence was given that by continuous absorption of very small quantities of oestrogen, certainly less than 3 to 4 μg. per day, fibromatogenic and epitheliotoxic actions on the endometrium were induced. In the present paragraph results with animals will be given in

TABLE I.—*Fifteen Castrated Females with Subcutaneously Implanted Tablets Containing 5 per cent α-oestradiol and 95 per cent Cholesterol ; the Same Tablet Implanted Subsequently into 3 or 4 Different Animals.*

Number of tablet and of implantation.	Duration of experiment. days.	Loss of weight of tablet mg.**	Absorption α-oestradiol calculated per day. μg.**	Uterine bleeding.	Uterine weight. gr.	Fibrous tumoral effect, in units.					Tumours of class 2 and 3 (14).
						Uterine.	Apical.	Dig. and abd. wall.	Spleen.	F.T.E.	
Tablet No. 3.											
First (3)*	112	2·4	0·9	6th week	7·3	0	0·5f	1	0	1·5	
Second (31)	125	0·4	0·1	5th „	10·5	3	2	0	0	5·0	2
Third (50)	111	—††	—	11th „	14·0	2	0·5f	2	0·5f	5·0	2
Tablet No. 4.											
First (4)	112	2·0	1·1	6th week	10·0	1	0·5f	2	2	5·5	2
Second (30)	124	1·0	0·4	No	4·2	0	1	0	0·5t	1·5	..
Third (51)	111	0·9	0·4	8th week	8·2	1	1	0	0	2·0	..
Fourth (65)	126	..	..	11th „	8·4	0·5t†	0·5f	0	1·5	..	..
Tablet No. 9.											
First (9)	114	2·1	1·0	No	3·8	0·5t	0·5f	0·5f	0·5f	2·0	..
Second (29)	124	1·4	0·5	„	6·2	0	0	0	0	0	..
Third (52)	110	0·6	0·2	6th week	16·0	3	3	0·5f	3	9·5	3
Fourth (66)	121	0·2	0·08	8th „	34·0	1	1	0	0	2·0	..
Tablet No. 10.											
First (10)	114	1·5	1·5	7th week	7·0	1	3	0·5f	0·5t	5·0	1
Second (28)	131	0·6	0·2	6th „	12·5	1	2	0	0	3·0	1
Third (53)	111	0·7	0·3	9th „	7·0	2	0·5f	0·5f	0	3·0	1
Fourth (67)	125	0·1	0·04	9th „	17·0	2	0·5f	2	0·5f	5·0	2

* No. of animal.

† Abbreviations : t = small nodular tumour ; f = fibrous strands ; F.T.E. = Fibrous tumoral effect. "Units" according to Lipschutz; see Lipschutz and Maass (1944).

†† Weight of tablet was at the end of experiment greater than at the beginning.

** Figures are of doubtful value. See text.

TABLE II.—*Castrated Females with Subcutaneously Implanted Tablets Containing 5 per cent Dipropionate of Estradiol and 95 per cent Cholesterol. Six Animals Autopsied after 119 Days of Estrogenic Action, and Six Animals Autopsied after 517 to 552 Days of Estrogenic Action.*

Number of tablet.	Duration of experiment. days.	Loss of weight of tablet. mg.**	Absorption α-estradiol calculated per day. μg.**	Uterine bleeding.**	Uterine weight gr.	Fibrous tumoral effect, in units.					Tumours of class 2 and 3 (14).	Histology of the uterus.
						Uterine.	Apical.	Dig. and abd. wall.	Spleen.	F.T.E.		
First implantation.												
11 (11)*	119	1·6	0·5	No	7·8	2	0·5f	0	0	2·5	1	Cyst. gland hyperpl.
12 (12)	119	1·1	0·3	3rd week	7·0	0·5t†	0·5f	2	3	6·0	2	„ „ „
27 (37)	126	1·0	0·3	No	3·5	0	0	0·5f	0·5f	1·0	..	„ „ „
28 (46)	125	3·5	1·0	11th week	5·0	0	0·5t	0	0·5f	1·0	..	„ „ „
29 (44)	129	1·4	0·4	No	5·0	1	0·5t	0	0·5t	2·0	..	„ „ „
31 (41)	125	1·4	0·40	12th week	5·3	0	0·5f	0	0	0·5	..	„ „ „
Second implantation.												
11 (56)	552	2·1	0·14	11th week	27·7	3	0·5f	0·5f	0	4·0	1	Invasive gland growth.
12 (57)	552	2·9	0·18	No	13·7	2	0·5f	0	0	2·5	1	„ „ „
27 (58)	552	2·3	0·16	No	6·5	0	0·5f	0	0	0·5	..	„ „ „
28 (59)	552	3·7	0·24	10th week	13·0	3	0·5f	0·5f	0·5f	4·5	1	Not examined.
29 (60)	552	5·1	0·33	No	3·5	0·5t	0·5f	0	0	1·0	..	Invasive gland growth.
31 (61)	517	2·3	0·17	4th week	4·0	0	0·5f	0	0	0·5	..	„ „ „

* Number of animal.
† Vagina open in all experiments.
†† Abbreviations: t = small nodular tumour; f = fibrous strands; F.T.E. = fibrous tumoral effect.
** Figures are of doubtful value. See text.

which the same small quantities of the oestrogens were active for 517 to 552 days in the same animal.

Comparative experiments were made with tablets containing 5 per cent of oestradiol dipropionate and 95 per cent of cholesterol Merck. Results are given in Table II.

There were in the first experiment six castrated guinea-pigs, which were necropsied 119 to 129 days after implantation of the tablet. Results were similar to those described in the former paragraph with free α-oestradiol. Fibromatogenic action was rather less pronounced than with the free hormones. But with the fibromatogenic action of oestrogens due allowance has to be made for variation.

In the second series in which tablets already used in the first one were again implanted and left in the body for no less than one and a half years, uterine growth was considerable. Uterine fibroids were also induced.

Microscopical examination of the uterus revealed in the series of long duration a condition fundamentally different from that seen at four months; there was besides cystic glandular hyperplasia of the endometrium also proliferation of glands infiltrating the myometrium, *reaching the serosa and even perforating it*. The lumen of the proliferating and infiltrating glands was sometimes cystic, sometimes small or absent. The nuclei of the cells of these structures seemed normal as to size and shape, but revealed hyperchromatism according to the statement of the pathologist. Contrary to the normal, the protoplasm was basophylic. There was no inflammatory reaction. The condition was similar in all the five animals examined. The pathologist, Prof. I. Mena, declared the condition to be that of an adenocarcinoma.

DISCUSSION.

The two comparative series described in the last paragraph show conclusively that small quantities of the oestrogen, which in experiments of four months' duration produced cystic glandular proliferation, elicit a different condition if allowed to act for 18 months; there was an invasion of proliferating glands into the myometrium. A similar picture has been described by Lipschutz (1937) in experiments in which the ovary was reduced to a small fragment. The duration of the experiments was of 30 to 33 months. The neoplastic and infiltrative growth of the glands was, in Lipschutz' experiments with ovarian fragmentation, coincident with those we have described in the last paragraph, with the exception that with ovarian fragmentation the invading glands did not reach the serosa and did not perforate it, as was the case in one of our animals. When comparing

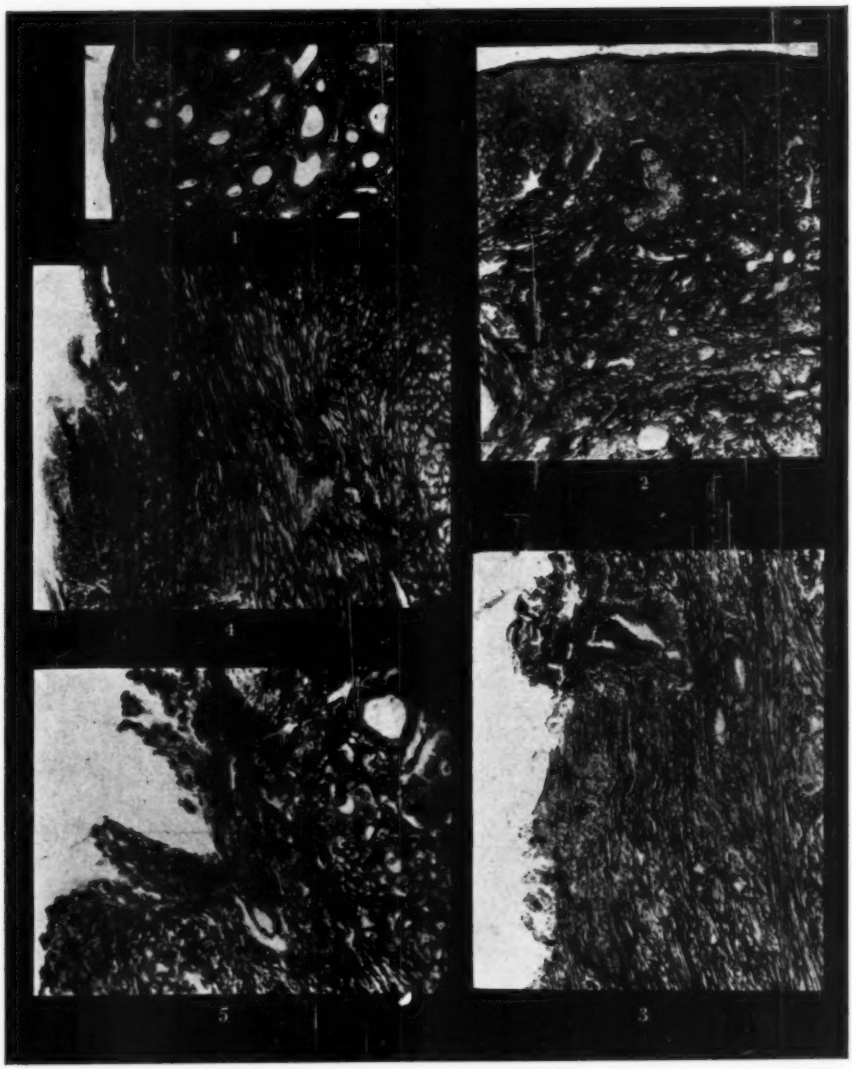
FIG. 1.—Proliferated uterine glands of different diameter, some cystic. The glands are invading the myometrium and come very near to the uterine serosa. Animal No. 57, Table II, 552 days of oestrogenic action. × 70.

FIG. 2.—Isolated gland, almost transformed into an epithelial cord, between the circular and longitudinal part of the myometrium. Animal No. 61, Table II, 517 days of oestrogenic action. × 135.

FIG. 3.—Above, proliferated uterine glands reach the serosa which is destroyed. Below, proliferation of the endothelium of the serosa. Animal No. 56, Table II, 552 days of oestrogenic action. × 135.

FIG. 4.—Proliferated uterine glands which have penetrated the uterine serosa and have mingled with the proliferated endothelium of the serosa. The same animal as Fig. 3. × 135.

FIG. 5.—Uterine glands in the myometrium near the serosa and proliferated endothelium of the serosa at higher magnification. The same animal as Fig. 3 and 4. × 300.



Riesco.

results with ovarian fragmentation and the present experiments the following statement can tentatively be made : With subcutaneously implanted tablets the infiltrative growth proceeded further than the ovarian fragmentation because in the latter case follicular phases, though prolonged, were occasionally interrupted by luteal phases.

So far as known to the author, there were no statements about similar atypical growth experimentally induced by oestrogens in the uterus of the guinea-pig besides those with ovarian fragmentation mentioned above. Another statement made by Lipschutz and his associates also deserves to be mentioned here (Lipschutz, Iglesias and Vargas, 1939). A guinea-pig having been injected for 8 months with oestradiol benzoate was found, 4 months later, to have a " precancerous " transformation of the endometrium in the upper third of the uterine horn. This latter observation of Lipschutz and others is so far the only one which may be quoted in favour of irreversible atypical proliferation of the uterine mucosa elicited in the guinea-pig by oestrogens and persisting after the withdrawal of the hormone.

Neoplastic malignant growth seems to be more easily elicited in the *cervix* of the uterus in the *mouse*, as shown by the work of Gardner and others (Gardner, Allen, Smith and Strong, 1938) and Allen and Gardner (1941). In the work of these authors the oestrogens—the benzoic ester of oestrone and oestradiol—were given by injection. The quantities injected were, when calculated per gram of body weight in the work of the American authors with mice, probably 20 times greater than in our work with guinea-pigs.

Another interesting aspect of our work may be mentioned here. The guinea-pig in which invasive growth of the uterine glands was obtained by ovarian fragmentation or by the prolonged action of hormones absorbed from subcutaneously implanted tablets in experiments of long duration, is highly resistant against the carcinogenic action of hydrocarbons. Lipschutz (unpublished) and other authors were unable to elicit epithelial tumours of the skin in the guinea-pig by painting with benzpyrene or methylcolanthrene. Iglesias and Riesco (unpublished) have worked with cylindric pellets of methylcholanthrene implanted subcutaneously into the abdominal cavity and into the brain of guinea-pigs ; there were no tumours in animals necropsied more than 500 days later. Shimkin and Mider (1941) were more fortunate with their strain of guinea-pigs in which malignant tumours were induced by injections of methylcholanthrene. Spontaneous tumours are also very rare in guinea-pigs, as shown by the work of Lipschutz (1941), Riesco and Schwarz (1944) and others.

SUMMARY.

Abdominal fibroids, glandular hyperplasia and adenomatous polyps were induced in 4 months in castrated female guinea-pigs with the absorption of less than 3 to 4 μg. of α-oestradiol free or esterified, per day administered by subcutaneously implanted tablets of the hormone mixed with cholesterol.

When the same, or smaller, quantities of the oestrogen were allowed to act for 18 months there was besides abdominal fibroids and cystic glandular hyperplasia also an invasive growth of the uterine glands in all the animals histologically examined.

The proliferating uterine glands which infiltrated the myometrium, reached the serosa and in one case perforated it.

Thanks are due to Prof. A. Lipschutz, to Dr. I. Mena and to Dr. Fuenzalida for helpful criticism, to Miss A. Contreras and Mr. S. Chaskel for technical help.

REFERENCES.

ALLEN, E., AND GARDNER, W. U.—(1941) *Cancer Research*, **1**, 359.
BISHOP, P. M. F., AND FOLLEY, S. J.—(1944) *Lancet*, i, 434.
DEANESLY, R., AND PARKES, A. S.—(1943) *Ibid.*, ii, 500.
FUENZALIDA, F.—(1944) *Rev. Canadienne de Biol.*, **3**, 366.
GARDNER, W. U., ALLEN, E., SMITH, G. M., AND STRONG, L. C.—(1938) *J. Amer. med. Ass.*, **110**, 1182.
IGLESIAS, R.—(1938) *Tesis Universidad de Chile* (Public. Med. Exper., No. 1).
LIPSCHUTZ, A.—(1937) *Gynéc. et Obstet.*, **36**, 407.—(1942) *Rev. Med. y Alim.* (*Chile*), **5**, 61.—(1941) *Arch. Pathol.*, **31**, 702.
Idem, BELLOLIO, P., CHAUME, J., AND VARGAS, L.—(1941) *Proc. Soc. exp. Biol. N.Y.*, **46**, 164.
Idem, IGLESIAS, R., AND VARGAS, J., JR.—(1939) *C. R. Soc. Biol., Paris*, **130**, 1536.
Idem AND MAASS, M.—(1944) *Cancer Research*, **4**, 18.
Idem, RODRÍGUEZ, F., AND Y VARGAS, L.—(1939) *C. R. Soc. Biol., Paris*, **130**, 939.
Idem, THIBAUT, R., AND VARGAS, L.—(1942) *Cancer Research*, **2**, 45.
RIESCO, A.—(1942) *Rev. Med. y Alim* (*Chile*), **5**, 158.—(1944) *Bol. Soc. Biol.* (*Chile*), **1**, 35.
Idem AND Y SCHWARZ, J.—(1944) *Ibid.*, **1**, 61.
SHIMKIN, M., AND MIDER, B.—(1941) *J. Nat. Cancer*, **1**, 707.

THE INDUCTION OF TUMOURS IN MICE OF THE R3 STRAIN BY 2-ACETYLAMINOFLUORENE.

L. FOULDS.

From the Laboratories of the Imperial Cancer Research Fund, Mill Hill, London, N.W. 7.

Received for publication June 17, 1947.

WILSON, DE EDS AND COX (1941) found that 2-acetylaminofluorene, given by mouth, induced a variety of tumours in rats, and Bielschowsky (1947) summarized the confirmations and extensions of their observations reported up to 1946. Armstrong and Bonser (1944) first demonstrated a similar action in mice, and recently (1947) described the induction of tumours in five inbred strains. The present paper records observations on one of the strains used by Armstrong and Bonser, and, in the main, confirms and amplifies their results.

METHOD.

Thirty-six male and 36 female mice of the R3 strain received food containing 2-acetylaminofluorene (AAF) and 49 male and 44 female mice from the same stock were kept as controls. Dobrovolskaïa-Zavadskaïa supplied the R3 mice which founded the line maintained in this laboratory by 45 generations of brother-sister matings since 1934. The exact ages of the mice were not known, but were believed to average about 3 months at the beginning of the experiment, at which time the sexes were separated.

The AAF was mixed with food prepared by grinding to powder the Thorley Rat Cubes used in this laboratory as the standard diet for rats and mice, or, during part of the experiment, was mixed with similar food supplied in powder form by the makers of the cubes. The concentration of AAF in the food was 0·03 per cent for 5 weeks and then 0·05 per cent for 20 weeks, the total period of administration extending over 25 weeks from October, 1945, to April, 1946. Thereafter the mice received the standard diet of Rat Cubes for the rest of their lives and the control mice received that diet throughout. All mice had an unrestricted supply of food and water. The experiment was terminated in the eighty-fourth week by killing the mice then surviving, namely 2 males which had eaten AAF and 3 control males.

Several attempts to control the localization of tumours were made on small groups of mice. During the third to sixth weeks of experiment 5 females of the AAF group and 5 control females were mated with vasectomized males and silk threads were tied in the pseudo-pregnant uterine horns to elicit deciduomata. In the twenty-second week of experiment, pellets containing 25 per cent of diethylstilboestrol (B.D.H.) in cholesterol and weighing 5 or 6 mg. were implanted subcutaneously in 9 females of the AAF group and in 10 control females. Disc-shaped tablets containing 5 mg. testosterone propionate (B.D.H.) compressed with 15 mg. calcium lactate were implanted subcutaneously, during the seventeenth and twentieth weeks of experiment, in 9 AAF males and in 10 control males. At various times 10 mammary tumours were excised from 7 AAF females with the primary object, dubiously achieved, of prolonging life to allow time for other tumours to develop.

RESULTS.

The heaviest mortality attributable with probability to the AAF occurred during the first few weeks of administration, when 11 males and 8 females died. Subsequently the toxic action of AAF was negligible.

No tumours grew in operation scars. The stilboestrol pellets shortened life, especially by causing pyometra. The testosterone tablets were harmless; their possible effect on the induction of bladder tumours is mentioned below. Granulation tissue, fibrosis and abscess were found in uterine horns where silk threads were tied but tumours were not recognized there.

"Spontaneous" tumours were found in 3 male control mice as follows: osteogenic sarcoma of the thigh, with secondary deposits in liver and lungs, in the forty-eighth week; hepatoma in the sixty-third week; and lymphoma in the sixty-eighth week. One female control had lymphoma in the sixtieth week, and many had mammary tumours as described below.

The AAF group provided single examples of (*a*) carcinoma of the small intestine with a secondary deposit in a mesenteric lymph node in the fifty-second week in a male which also had a tumour of the bladder, and (*b*) in the eighty-second week, again in a male with a tumour of the bladder, juxta-renal tumours of lymphoid structure.

The tumours requiring fuller mention were mammary tumours, tumours of the bladder and hepatoma. Table I shows the times of observation of the first tumour of each kind, the number of mice ("effective total") surviving at those times, and the number of tumours. Only tumours visible to the naked eye are included.

TABLE I.

Type of tumour.	Earliest tumour (weeks of experiment).	Sex.	AAF mice. Effective number of mice.	AAF mice. Number of tumours.	Control mice. Effective number of mice.	Control mice. Number of tumours.
Mammary	11	♂	25	0	45	0
		♀	28	17	42	22
Bladder	39	♂	23	12	44	0
		♀	13	0	19	0
Hepatoma	60	♂	9	2	41	1
		♀	4	4	6	0

Mammary tumours.

Mammary tumours were found in 60·7 per cent of the effective total of female AAF mice and in 52·4 per cent of the control females. The times of observation ranged from 11 to 61 weeks, with an average of 35·5 weeks in the AAF group, and from 12 to 74 weeks with an average of 40·5 weeks in the controls. Since the exact ages of the mice and their reproductive history before the experiment began are not known, the evidence for an enhancement of mammary tumour development is not convincing. Stilboestrol had no apparent effect on the incidence of tumours, but the number of animals tested was small and soon depleted by deaths from pyometra, and the pellets were implanted at possibly too late a stage of the experiment.

Tumours of the bladder.

Tumours of the bladder were found in 12 male mice which received AAF, but not in females which had AAF or in male or female controls. The first of the tumours was found in the thirty-ninth week of experiment and the last in the eighty-second week. The average time was 50·7 weeks, but 8 of the tumours were found from the thirty-ninth to forty-sixth weeks. Only 1 tumour occurred in an effective total of 8 mice carrying testosterone tablets; 6 of the mice survived from 58 to 64 weeks without tumours. The incidence as a percentage of the effective total was 12·5 for AAF males with testosterone tablets, 73·3 for AAF males without testosterone, and 52·2 for all AAF males.

Most of the tumours were intravesical, pedunculated or sessile papilliferous tumours of transitional- or squamous-cell types, and conformed with the descriptions of Armstrong and Bonser (1944), who distinguished benign and malignant forms but observed no extra-vesical extension. Two tumours of my series extended outside the bladder and disseminated in the abdominal cavity. These two tumours and one which was wholly intra-vesical were transplanted, and consideration of the structural and biological features of the tumours of the bladder is deferred until the transplanted tumours have been further studied.

Hepatoma.

The first hepatoma was found in a female of the AAF group killed in the sixtieth week. Three AAF females then survived, and hepatoma was found in each of them during the sixty-second week; one of them had also a mammary tumour. Hepatoma was present in the 2 AAF males which were killed to

terminate the experiment in the eighty-fourth week, and in 1 male control which died in the sixty-third week.

The tumours, of trabecular type, were unexceptional in structure and were judged benign. Transplantation of 3 tumours into a total of 22 R3 and 4 hybrid mice was unsuccessful.

DISCUSSION.

Armstrong and Bonser administered 2-acetylaminofluorene twice weekly by stomach-tube until the mice seemed unlikely to survive longer. The present experiment shows that 2-acetylaminofluorene was effective when administered with the food for a limited period of 25 weeks, although the induced tumours were not found until 14 to 59 weeks after the drug was withdrawn. The outstanding result was the growth of tumours of the bladder in about half the male mice which survived 9 months or longer from the beginning of the experiment.

The sites of predilection for tumours induced by 2-acetylaminofluorene include liver, bladder, breast, external auditory canal and intestine. The distribution of tumours amongst these and less common sites depends on the species and on the strain of the animals used (Bielschowsky, 1946, 1947; Armstrong and Bonser, 1947; Dunning, Curtis and Madsen, 1947; Harris, 1947). Harris concludes that "the site at which tumours may be induced by fluorene compounds is probably determined by the genetic constitution of the rat employed." This statement, though true, is not a definitive explanation. The genetic constitution determines how particular cells react to particular stimuli; it remains to determine what reactions are important in the production of tumours by acetylaminofluorene and how the reactions are variable in kind or degree. The importance of the genotype is accepted; the urgent task is to elucidate its phenotypic expression.

Carcinogenic hydrocarbons affect tissues remote from the site of application and increase the incidence of certain tumours, notably tumours of the breast, long adenomas and leukaemia. Many observations, summarized recently by Engelbreth-Holm and Rask-Nielsen (1947), suggest that the action is, essentially, an acceleration of spontaneous tumour development. Some actions of acetylaminofluorene may be attributable to acceleration of a spontaneous disease as, for example, the increase of mammary tumours recorded by Armstrong and Bonser. In my experiment the evidence for an increase in the naturally high incidence of breast tumours in R3 mice was not decisive, and acetylaminofluorene did not accelerate or increase the incidence of lymphoma or sarcoma which occur, uncommonly, in normal R3 mice. The unequivocal effect of aminofluorene was the growth of tumours of the bladder which are not found in normal controls and which, as yet, have not been induced in mice by other carcinogens (Bonser, 1947). In some strains of rats, acetylaminofluorene notably induces carcinoma of the external auditory canal, previously unrecorded. These and other comparable observations show that tumours induced by acetylaminofluorene do not grow preferentially in tissues prone to develop "spontaneous" tumours and are not accelerated "spontaneous" tumours. The observed distribution of tumours probably depends, to an important degree, upon a time-factor in the response to acetylaminofluorene. According to Armstrong and Bonser, bladder tumours and hepatomas are about equally frequent in CBA mice, and a table in their earlier paper (1944) shows that, on the average, the hepatomas appeared earlier than the bladder tumours. In R3 mice, however,

hepatoma is much less frequent and, as my observations show, develops later than tumours of the bladder by about 20 weeks—a substantial fraction of the life of a mouse. Consequently, intercurrent diseases, mammary tumours in females or bladder tumours in males kill most R3 mice before the response of the liver to aminofluorene is manifested by the growth of hepatoma. Extreme differences in the incidence of a tumour, as of hepatoma in different strains of rats (Bielschowsky, 1946), do not necessarily indicate qualitatively different responses to acetylaminofluorene; they are as well accounted to different *rates* of response. Some geneticists maintain that the processes directed by allelomorphic genes differ only in speed. Speed of reaction is probably one of the most important expressions of the genetic factors which govern the distribution of the tumours induced by acetylaminofluorene. The high and relatively early incidence of mammary tumours in female R3 mice presumably reduces the opportunities for other tumours to become evident, but it does not account for the complete absence of bladder tumours from the females in my experiment, or for the lower incidence in females compared with males in the 5 strains studied by Armstrong and Bonser. A sex factor evidently conditions the response of the bladder to acetylaminofluorene. The possible inhibitory action of testosterone on the induction of bladder tumours needs checking on larger numbers of mice. Bielschowsky (1944) and others observed a sex difference in the incidence of hepatoma in rats. It is concluded that the factors which decide the observed distribution of tumours include genetically-determined rates of response of different tissues and a sex factor which, possibly, operates through hormones.

SUMMARY.

Acetylaminofluorene, administered in the food for 25 weeks to mice of the R3 strain, induced tumours which were found from 14 to 59 weeks after withdrawal of the drug. Tumours of the bladder grew in about half the male mice which survived 9 months or longer from the beginning of the experiment. Testosterone seemed to inhibit the induction of bladder tumours in males and none grew in females. Hepatoma was found in the males and females which survived longest. A discussion of the selective localization of aminofluorene tumours leads to the conclusion that the deciding factors include genetically-determined differences in the rates of reaction to the drug of various tissues and, possibly, sex-hormones.

ACKNOWLEDGMENT.

I am indebted to my colleague, Mr. H. G. Crabtree, for the preparation of the acetylaminofluorene.

REFERENCES.

ARMSTRONG, E. C., AND BONSER, G. M.—(1944) *J. Path. Bact.*, **56**, 507.—(1947) *Ibid.*, **59**, 19.

BIELSCHOWSKY, F.—(1944) *Brit. J. exp. Path.*, **25**, 1.—(1946) *Ibid.*, **27**, 135.—(1947) *Brit. Med. Bull.*, **4**, 382.

BONSER, G. M.—(1947) *Ibid.*, **4**, 379.

DUNNING, W. F., CURTIS, M. R., AND MADSEN, M. E.—(1947) *Cancer Res.*, **7**, 134.

ENGELBRETH-HOLM, J., AND RASK-NIELSEN, R.—(1947) *Ibid.*, **7**, 129.

HARRIS, P. N.—(1947) *Ibid.*, **7**, 88.

WILSON, R. H., DE EDS, F., AND COX, A. J.—(1941) *Ibid.*, **1**, 595.

FORCED ACTIVATION AND EARLY DETECTION OF THE MILK-BORNE AGENT OF MAMMARY ADENOMAS IN MICE.

B. D. PULLINGER.

From the Laboratories of the Imperial Cancer Research Fund, Mill Hill, London, N.W. 7.

Received for publication June 9, 1947.

THE presence and activity of the milk-borne agent of mammary tumours of mice discovered by Bittner (1936) are detectable only by the growth of a mammary tumour. No other criterion of its presence is yet generally accepted, though an opinion is widely held that certain localized nodular adenomatous mammary hyperplasias are an expression of the combined activity of the milk-borne tumour agent and a mammogenic hormone. Focal or nodular adenomatous hyperplasias in the mammae of dealer's mice were first described by Apolant (1906) and their cause investigated by Haaland (1911). Haaland inquired but failed to decide whether the nodules (Fig. 1 and 2) were tumours from the beginning, or whether they were the basis from which tumours develop. Similar nodules occur in some inbred strains of mice. Thus Gardner, Strong and Smith (1939) found their incidence to be greatest in mice of the high cancer strains D and C3H, and intermediate in the low mammary tumour strain CBA and high mammary tumour strain A. Objections to accepting nodular hyperplasia as evidence of activity of the milk-borne tumour agent derive from two sources. In the first place these nodules have occasionally been found in strains that do not develop mammary tumours. Gardner *et al.* found two nodules in one mouse out of nine of the N strain in which mammary tumours had never occurred. Also when efforts have been made to exclude the milk-borne agent from high cancer strains by cross-suckling, nodules have nevertheless been found. Thus Bittner, Huesby, Visscher, Ball and Smith (1944) examined the mammae of fostered breeding females (fostered by cancer-free maternal stock) from high cancer strains A and C3H. All the females had given birth to at least three litters and ranged from 11 to 16 months old, thereby providing suitable conditions for pathogenic activity of the milk-borne agent. Nodules were found occasionally, though never more than one to a gland. Thus nodules were still present though in smaller numbers when efforts were made to exclude them than in mice which had been suckled on their own cancer-prone mothers. This evidence indicated that nodule incidence is not eliminated by cross suckling with the same regularity that is attainable in regard to tumour incidence. Huesby and Bittner (1946) record that they found no nodules in the mammae of 6 virgin, and ¼ per gland in 5 out of 12 breeding Zb females; none in 6 virgin and ¼ per gland in one out of 12 breeding Strong AX females; none in 10 hybrid virgin females and ¼ per gland in 2 breeding females of the F.1 generation derived from these two strains, both deprived, previous to crossing, of the milk-borne agent.

They state also that only 7 nodules were encountered in 197 glands of 51 animals that were genetically susceptible to and had sufficient hormonal stimulation for mammary carcinoma, but did not develop it because of the absence of the milk influence. It seems that a possible reduction in the amount of the milk-borne tumour agent within a strain has to be taken into consideration as well as complete absence. The necessity then arises for scrutinizing every mammary gland.

More recent data derived from control observations to experiments on the induction of mammary nodules by methylcholanthrene in the absence of the milk agent (Kirschbaum, Williams and Bittner, 1946) suggest that the first objection may have been overcome to some extent. These authors give in their Table I particulars of nodule incidence or absence in three inbred strains. Their Zb subline lacking the milk-borne agent was derived from the high mammary cancer line Z (identical with C3H, Bittner *et al.*, 1944) by cross suckling. The mammary glands of 123 breeding females from this Zb subline were found to be free of hyperplastic nodules. None were found in 90 C57 (black) nor in 60 NH breeding females. Neither the subline nor these last two mammary cancer-free strains had developed any mammary tumours in the course of breeding. If all the mammae of the Zb subline were examined by the authors,

---

FIG. 1.—Spontaneous nodular hyperplasia in a mammary gland of a virgin female mouse aged 14 months. × 3.

FIG. 2.—Spontaneous nodular hyperplasia in a mammary gland of a virgin female R3 mouse (carrying the milk-borne agent), aged 13 months. × 14.

FIG. 3.—Hyperplasia induced with 400 Y oestrone in one dose in a spayed R3X virgin female (lacking the milk-borne agent). × 7.

FIG. 4.—Hyperplasia and cystic distension induced by 800 Y of α-oestradiol in two doses in a spayed R3X virgin female (lacking the milk-borne agent). Note tendency to nodular shape. × 14.

FIG. 5.—Complete regression of hyperplasia induced by 800 Y oestradiol in spayed R3X female (lacking the milk-borne agent) at 6 months old. × 4.

FIG. 6.—Regression of hyperplasia induced by 800 Y oestradiol in spayed R3 female (carrying the milk-borne agent), and persistence of areas of nodular hyperplasia (adenomas) at 6 months old. × 4.

FIG. 7.—Early response in a mammary gland of a spayed virgin female mongrel mouse to 800 Y oestradiol, typical also of C57 and Strong A strains. × 4.

FIG. 8.—Early response in a mammary gland of an F.1 hybrid spayed virgin female of C57 x R3X (lacking the milk-borne agent). Note focal hyperplasia in part only of the gland. × 4.

FIG. 9.—Incomplete involution at edges of a mammary gland in a spayed R3X virgin female, aged 6 months, after the same treatment as mice in Fig. 5 and 6. × 4.

FIG. 10.—Incomplete involution shown by "ghost" acini in a mammary gland of a male mouse 5 months after hormone stimulation. (See Table II.) × 4.

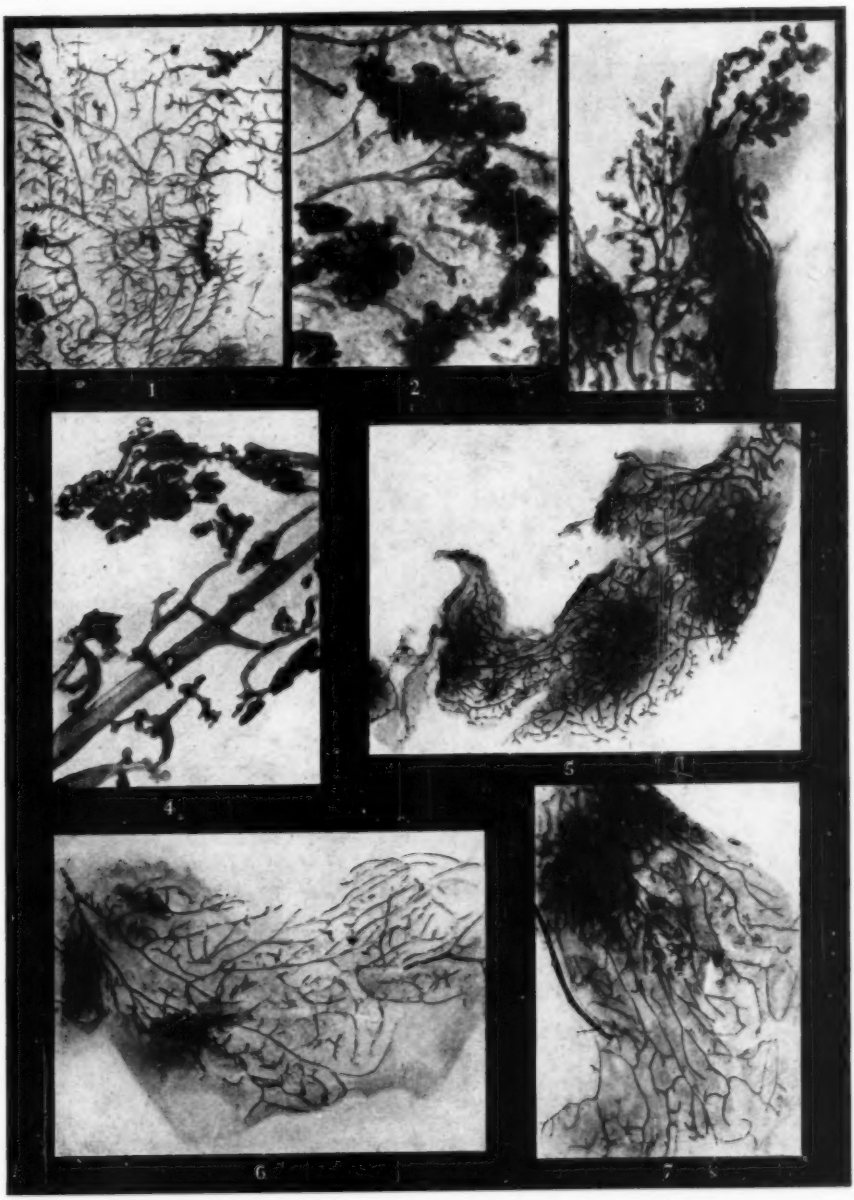
FIG. 11.—Secondary foci of proliferation in an adenoma forced into activity by oestrogen in a spayed R3 virgin female mouse, aged 6 months. × 7.

FIG. 12.—Secondary focus of proliferation in an adenoma forced into activity in an R3 male mouse, aged 6 months. × 7.

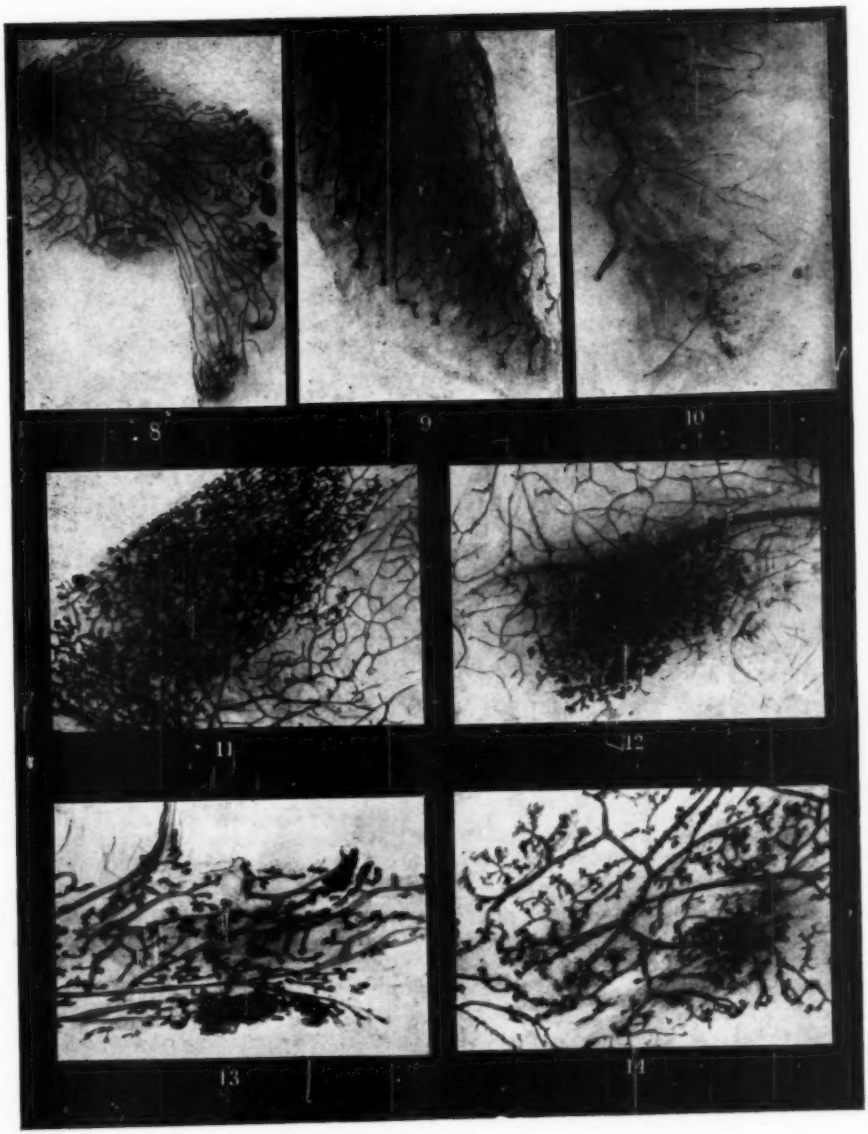
FIG. 13.—Nodular hyperplasia in a mammary gland 9 weeks after spaying an R3 virgin female and after the first of two doses of oestradiol, showing the combined response to hormone and agent. × 7.

FIG. 14.—Nodular hyperplasia in a mammary gland 3 weeks after spaying an R3 virgin female and treating with 200 Y oestrone, showing combined response to hormone and agent. × 4.

All figures are microphotographs of mouse mammae stained in bulk in dilute haematoxylin and mounted in bulk.



Pullinger.


8
9
10
11
12
13
14

there were 1,230 mammae without hyperplastic nodules. This may have been due to a more successful transfer of young mice at the time of cross suckling than in the previous experiment, or to some cause for which we have not at present got the clue. The former discrepant results (Bittner *et al.*, 1944 ; Huesby and Bittner, 1946) have to be remembered, although subsequent observations (Kirschbaum *et al.*, 1946) provide convincing evidence that hyperplastic mammary nodules do not occur in the absence of the milk-borne tumour agent.

The second source of objection to accepting nodular hyperplasia as evidence of activity of the milk-borne agent acting alone is that certain observations, which will be referred to again later, suggest that similar cellular proliferations with acinus formation are stimulated by oestrogenic hormones alone.

The problem of the exact origin of the hyperplastic nodules is of great importance for several reasons. These focal acinar proliferations precede malignant tumours in time of appearance and are often more numerous. They might thus provide an alternative and earlier means of detecting activity of the tumour agent. They may also represent the earliest sign of tumour growth before it can be described as malignant. If so they would provide material enabling one to determine which influences are causative and which are encouraging, in the sense in which the term is used by Rous and Kidd (1941) and Mackenzie and Rous (1941) in the development of malignant tumours. They would undoubtedly also provide a test in any method devised for analysing the factors which favour initial pathogenic action of the agent. The discrepant results (previously referred to) concerning their incidence often suggest that they may be more sensitive criteria of the presence of the agent. If the nodular hyperplasias are to be used for any of the purposes suggested, it must be proved beyond question that they are in fact dependent for their origin and continued existence on the presence of the milk-borne tumour agent, and that the nodules, or some of them, are but an early stage of eventual malignant tumours. Other possible causes of focal cell proliferation such as hormones must be excluded. The possibility has also to be considered that mice of high cancer strains carry more than one formative milk-borne stimulus for the mammary gland. One agent might be the cause of frankly malignant tumours and another the cause of nodular hyperplasias. It appears more probable, however, that one and the same agent is responsible for both types of tumour, benign and malignant, but that its virulence varies. Greater virulence would be understood as the capacity to stimulate the mammary gland cells to multiply and invade or metastasize within the life of the mouse ; less virulent variants would cause merely focal proliferations. The assumption has been made for present purposes that only one milk-borne formative stimulus for the mammary gland is transmitted by mice. Whether the cell proliferations which it produces are benign or malignant depends upon the combination of its " virulence " with encouraging or deterrent forces.

The experiments here recorded are concerned only with the origin, provocation and continued existence of the adenomatous hyperplastic nodules. It was found possible to stimulate the appearance of nodules artificially with measured limited amounts of hormone at an earlier date than that at which they become detectable spontaneously. It was also possible to distinguish responses in the mammary gland due to hormone alone. Responses to other formative stimuli were considered.

Mammae of female and rudimentary mammae of male mice respond to

two known classes of formative stimuli in adult life at a time when embryological impulses have ceased to act. These two known classes are firstly, sex hormones, both natural and synthetic, and secondly, a milk-borne tumour agent having the general characters of a virus (Bittner, 1945, and Andervont, 1945). Other formative impulses capable of acting on the mammary gland probably exist in nature. Carcinogenic hydrocarbons have been found under certain conditions to induce mammary tumours in the absence of the milk-borne tumour agent as judged by the absence of palpable mammary tumours in the control groups of mice (Shimkin, 1945), a criterion which must now be called in question as the sole evidence of presence of the agent. Hyperplastic nodules undergoing metaplasia were found by Kirschbaum *et al.* (1946) after treatment with methylcholanthrene in the absence of the agent as judged by the absence of nodules in untreated controls. Local damage to mammary epithelium leading to regeneration is also a stimulus to proliferation (Coen, 1888). There may be others, for example "chronic irritation," though Haaland (1911) and Woglom (1945), who sought by observation and experiment respectively to reveal such a cause, were both unable to do so.

A preliminary step was required to find whether oestrogenic hormone alone (in the entire absence of milk-borne agent) is able to stimulate focal or nodular hyperplasia similar to or distinguishable from that due to the agent, and whether, if it occurs, such hyperplasia is capable of persisting in the absence of hormone or other formative stimulus.

It is impossible to solve the problems as they are related to the milk-borne tumour agent in the entire absence of hormone, because the agent requires the presence of hormone to become active in respect of tumour cell proliferation (Lathrop and Loeb, 1916; Shimkin, 1945).

In order to determine the action of hormone alone it was advisable to use mice as much alike as possible. The inbred strains were tested on the assumption that the mammary gland response of a physiological kind or quality would be more likely to be similar within a strain than would the responses of a number of outbred stock mice. The opinion has been expressed and some data relating to male mice provided in support of it that mammae of various strains respond differently to oestrogenic hormones in respect of acinus development (Lacassagne, 1934; Gardner, Diddle, Allen and Strong, 1934; Gardner, Smith and Strong, 1935; Burrows, 1936; Bonser, 1936). The results of these authors indicated also that acinus formation was an occasional feature only, and was independent of any association with proneness to mammary cancer (Burrows, 1945). Variation in anatomical structure of the mammary glands of different strains has been reported by v. Gulick and Korteweg (1940). A difference, which is inherited, in susceptibility to pathogenic activity of the milk-borne tumour agent exists in inbred strains (Bittner, 1939; 1943). For all these reasons it was necessary to assume and seek for a strain difference. Strains examined for response to hormone alone were R3X* males and females deprived of the agent by cross suckling; C57 (black) females; Strong AX females and males; Simpson females. The last are an inbred strain in which pure line breeding has been once interrupted since 1937 and replaced by mating of distantly related progeny. Though derived from an original strain with a high incidence of mammary

* An X after a mouse strain indicates in this paper that the strain had been freed from the milk-borne tumour agent.

cancer, breeding females have never developed mammary cancer in these laboratories.

Before testing the high cancer line R3, sublines deprived of the milk-borne tumour agent were bred. Strong AX sublines have been maintained in these laboratories since 1942. Milk-borne tumour agent had to be excluded because the presence of this second formative agent might confuse results due to responses to hormones alone. All the virgin females tested were segregated from males at the time of weaning (3 to 4 weeks old), and were spayed between the 49th and 56th days of age. These steps were taken to avoid changes due to pregnancy or pseudo-pregnancy, and to have in action, so far as possible, known measured doses of hormone. The same procedure excluded changes due to failure of involution of lactating or partially stimulated mammary glands. The responses to single and repeated doses of ovarian hormones were examined at various intervals after application as described under experimental methods.

The existence of a strain difference in response to oestrogens which was independent of the presence of milk-borne tumour agent was confirmed. The response was atypical in R3X and Simpson mice and will be described in more detail under results. Focal hyperplasia of acini was found widespread in all the R3X and Simpson mammae after treatment with the oestrogens, oestradiol and oestrone (Fig. 3 and 4). It did not persist indefinitely in the absence of the hormone. A similar response was obtained in R3 mice (containing the milk-borne tumour agent), but while the hormone conditioned response regressed and finally disappeared in the absence of further artificial stimulation in spayed mice, there remained in these mammae irregularly scattered areas of focal nodular hyperplasia which did persist in the absence of further hormonal stimulation (Fig. 5 and 6). Both in their origin and continued existence these nodules are considered to have been caused by the milk-borne tumour agent, though their origin was conditional on the presence of the hormone. They will subsequently be referred to as adenomas. A similar hormonal response was obtained in C3H mice giving rise to focal hyperplasias, but as this strain was not freed from the milk-borne tumour agent, it is uncertain, though probable, that the response to hormone alone would have given this atypical reaction. The C3H mice also developed adenomas which were maintained in existence when no further ovarian hormone was being supplied, and no evidence of any naturally secreted oestrogen was obtained from vaginal smears. A few other strains were examined (Table III).

EXPERIMENTAL METHODS.

Choice of mice.

Inbred R3 (Paris) mice were used. Some were derived from pure lines, others from random breeding within the strain. The incidence of spontaneous nodules of hyperplasia in the mammae in 26 virgin females segregated from males had previously been found to be 100 per cent after the age of 8 to 9 months. Malignant tumour incidence in the same series was 69 per cent. Nodules in this strain are extremely numerous in both virgin females and breeders.

R3X sublines free from the milk-borne tumour agent were bred by foster nursing an original family of 2 females and 1 male on a C57 (black) mother from the moment of birth. From one of these females several sublines were obtained. All the R3X mice used were the progeny of brother and sister matings. A

record was kept of the relationships of every mouse of these sublines so that, if evidence of the presence of milk-borne tumour agent subsequently reappeared, the progeny liable to be affected could be traced. From every litter containing more than one female, one or two were used for testing the hormone response while the remainder were allowed to breed. This breeding provided further mice for testing, and also controls to prove the absence of the milk-borne agent. The same breeding females also provided controls for all the male mice that were treated. Altogether, 44 breeding females reached the average tumour age (8½ months for this strain); of these 24 were force bred owing to the circumstance that they failed to rear or destroyed their young, a habit to which many R3 females are prone. In three years no tumours were seen. The average age at death of the breeding females was 14 months. Two hyperplastic nodules were seen in two breeding females that failed to rear any litters. No others were seen in the breeding stock.

Operative treatment.

Virgin females were spayed at approximately 56 days old. The exact age was always known, but not the previous oestrus record, if any. Many R3 and R3X females were found, by the vaginal smear technique, to have started oestrus cycles before this time. The treated males varied from 5 to 9 weeks old. Twelve R3 males were castrated.

After removal of hair with a depilatory from the abdominal surface, the females were anaesthetized with 0·3 ml. of a 3 per cent suspension in distilled water of bromethol, except when Strong A mice were to be operated on. The majority of Strong A mice when two months old or over failed to survive the combination of this anaesthetic and ovariectomy. Their survival could only be assured after they had reached the age of 2 months, if ether were used. The ovaries of all mice were removed by the abdominal route; they were excised together with about ⅛ in. of their oviducts (uterine horns). Successful removal of all local oestrogen-producing tissue was judged at autopsy by complete atrophy of oviducts and uterus. Vaginal smears were made from some of the females for 2–3 weeks before they were killed. It was necessary to examine the former site of uterus and oviducts in every mouse, because if the tissues were not excised as described, cysts containing clear fluid sometimes developed at the cut ends of oviducts after subsequent oestrogen treatment. When this was the case atrophy of uterus and oviducts was never complete, and the impression was gained as the result of much experience of mammary gland changes that these cysts were themselves secreting oestrogen. This difficulty was soon overcome at a time when oestrus records were not being made in spayed mice, so the matter was never settled. When ovariectomy and removal of approximately the upper ⅛ in. of oviducts was complete, no cysts were seen at autopsy 16 to 20 weeks later. The oviducts became very slender tubes (brownish in R3 and R3X mice) and the uterus was greatly shrunken. Oestrus cycles ceased in all those mice which were tested and did not reappear. Any mice that had developed intra-abdominal cysts or ligature abscesses, thereby possibly concealing cysts, at the cut ends of the oviducts, or whose oviducts had not undergone atrophy at the 16th to 20th weeks of experiment, were discarded. At earlier dates atrophy may be incomplete, varying in degree with the original dose of hormone.

Hormone treatment.

In accordance with a recommendation of Mixner and Turner (1943) treatment of the females with hormone was begun within 24 hours of the spaying (usually 18 hours). A measured dose was delivered from a graduated 0·2 ml. pipette on to the skin of the whole length of the back after clipping off the hair. The hormones were dissolved in acetone, a 0·01 per cent solution for the smaller doses (10–100 Y) and a 0·1 per cent solution for the larger ones (200–800 Y). The larger doses were delivered to females and males in divided doses over a period of 2 to 4 hours in hourly applications in the hope thereby of increasing absorption. For the same reason a final application of acetone alone was given. The method is recognized as being inaccurate and of comparative value only as regards quantitative dosage. The purpose in using it was to give a limited measured amount of hormone within at most a few hours in order to obtain a limited response rather than a cumulative one due to prolonged irregular absorption as from oily solutions or pellets. The ideal is a watery solution injected intravenously, but at the time experiments were begun none was available in sufficient strength to give large doses.

Preliminary experiments had indicated that two large arbitrarily chosen doses of oestradiol of 400 microgrammes each applied at 20 days' interval were effective in stimulating combined pathological activity of milk-borne tumour agent and hormone. Later experiments showed this dose to be unnecessarily large for this purpose in R3 mice (See Table I). It was adhered to in the present

TABLE I.—*Comparison of State of Involution and Adenoma Incidence in Mammae in Spayed Virgin* Female *Mice After Limited Treatment with Oestrogen.*

Mouse strain.	Dosage in microgrammes: α-Oestradiol. 1st dose.	α-Oestradiol. 2nd dose.	Oestrone. Single dose.	Number of mice with involution complete at— 16 weeks of experiment.	20 weeks of experiment.	28–31 weeks of experiment.	Number of mice. Total.	With adenomas.
R3	400	400	—	21	1	6	28	28
	—	—	50	—	9	—	9	9
	—	—	30	6	—	—	6	1
	—	—	10	—	6	—	6	0
R3X	400	400	—	7 out of 12	8 out of 8	—	20	None

X after the strain denotes absence of milk-borne tumour agent.
— denotes no test was done.

Total mammae examined, 690.

series of experiments because it seemed adequate and not too large to provoke similar results in R3 x C57 (F.1) hybrids treated in the same way. It has been retained as the maximum dose for provoking the agent, and for judging and comparing hormone response alone in different strains and hybrids. Experiments using a descending scale of dosage are in progress. The results indicate that responses in females vary quantitatively with dosage and qualitatively with strain and, within the strains, with ages younger than 49 to 56 days old. For the purpose of the present experiment most of the females received two doses of 400 microgrammes at 20 days' interval. Some of the males were treated with three doses of oestrogen and one of progesterone (see Table II), on an assump-

TABLE II.—*Comparison of State of Involution and Adenoma Incidence in Mammae of* Male *Mice After Limited Treatment with Ovarian Hormones.*

Mouse strain.	Dose of oestrogen* in microgrammes. 1st dose.	2nd dose.	3rd dose.	Dose of progesterone in microgrammes.	Number of mice with involution Complete at— 24 weeks of experiment.	28	Number of mice. Total.	With adenomas.
R3	400	400	400	1,500	5	25	30	28
	400	400	400	—	10	—	10	10
	400	400	—	—	11	—	11	4
R3X	400	400	400	1,500	—	18†	18	None
	400	400	400	—	10	5	15	None

X after the strain denotes absence of milk-borne tumour agent.
— denotes that no test was done.
* α-oestradiol or ketohydroxyoestrin.
† 6 contained ghost acini (see Fig. 10).

Total fat pads examined, 840.

tion which proved to be incorrect so far as the R3 strain is concerned—that the progesterone would be required to bring about lobular-alveolar development in male mammae. It did, however, aid mammary outgrowth and differentiation probably in the way indicated by Mixner and Turner (1943). Details of oestrogens and dosage are given in Tables I, II and III.

TABLE III.—*Preliminary Test of Early and Late Responses to Oestrogen in Other Strains of Female Mice.*

Strain.	Dosage of oestradiol in microgrammes. 1st dose.	2nd dose.	Early response.	Late result at 16 to 20 weeks. Total mice.	Number with adenomas.
C3H	400	400	Lobular-alveolar (patchy)	10	10
Strong A	400	400	Normal	10	None
Simpson	400	400	Lobular-alveolar (widespread)	7	None
C57 (black)	400	400	Normal	—	—
C57 x R3X F.1	400	400	Half gland normal; half lobular-alveolar (Fig. 8)	27	None
R3 x C57 F.1	400	400	—	19	20
Mongrels (Liverpool)	400	400	Normal in 2 mice; lobular-alveolar (widespread in 1; focal in 7)	—	—

— indicates no observation was made.

Examination of mammae.

Mammae were examined after the first or second application of hormone at intervals which varied according to the purpose of the experiment. All mammae were dissected out and fixed flat together with the skin on sheet cork. In several mice responses were observed also *in vivo*. This is possible because in R3 and R3X females the large double dose (800 microgrammes) invariably stimulated the 2nd mammary glands to grow out into the interscapular fat on the back. This was seen in 15 out of 15 R3X females. If mice which have been treated with oestrogen at 20 days' interval are anaesthetized 4 days to 3 weeks after the second dose and a small incision is made in the mid line, it is possible to see this response with the naked eye, with a loupe or magnified a few times with a

binocular dissecting microscope after parting the cut edges of skin by gentle blunt dissection. Of the strains examined only R3, R3X and Simpson females have been found to grow out regularly in this way. The response is visible owing to its atypical nature in respect of a milky secretion which renders ducts and clusters of acini visible. Mice examined *in vivo* in early stages and all others were finally killed. All 10 mammary glands (or fat pads in males) were stained in bulk, cleared, inspected, and whole mounts made of representative mammae or those showing special features.

The method of staining in bulk differed little from that described by Gardner (1934). After 4 days' fixation in Bouin's fluid the mammae were dissected off from the skin, muscle and covering fascia with the aid of a dissecting microscope magnifying 7 and 14 times. Nerve fibres were removed and some of the large blood vessels. These stripped mammae were then immersed overnight in 50 per cent alcohol, and next stained for about 4 hours in 1 part of Ehrlich's acid haematoxylin freshly diluted with 3 parts of a 2 per cent aqueous solution of acetic acid. After differentiating for about 10 minutes in acid alcohol (3 per cent HCl in 50 per cent alcohol) the stained glands were rendered blue or blue-black in several changes of 50, then 70 per cent alcohol for as many hours or days as were required to develop a deep colour. They were next dehydrated and cleared in the usual manner. While in the first change of clearing agent (xylol) notes were made by the author after examining all 10 mammae or fat pads with the binocular dissecting microscope. Some were selected for making permanent mounted preparations and for storage in paraffin. The remainder were discarded.

RESULTS.

R3X females deprived of their ovaries and of the milk-borne tumour agent.

Twenty days after the application to the skin of 400 microgrammes of keto-hydroxyoestrin, 0·1 per cent dissolved in acetone, the duct system had grown out and branched extensively (Fig. 3). The ducts were distended and filled with milky secretion, which rendered the main branches and many smaller ones visible to the naked eye in all 10 mammary glands. There was also a pseudo-lobular alveolar differentiation with great multiplication of acini arranged in clusters in all 10 mammary glands. The large clusters could also be seen macroscopically as milky white lobed structures. Many of the acini composing them were ballooned out with secretion (Fig. 3). A similar response was given by the maximum dose of 400 Y of α-oestradiol repeated at 20 days' interval (total 800 Y) in 15 out of 15 mice which were examined *in vivo*. The most noticeable difference after the larger dose was outgrowth in every mouse into interscapular fat pads and possibly more secretion (Fig 4).

These responses are atypical and pathological. They differ from changes in the normal female mouse in oestrus (Cole, 1933; Turner and Gomez, 1933) and from responses in normal mice artificially treated with oestrogens (Turner and Gomez, 1934) in that lobular-alveolar differentiation and secretion occur in addition to duct outgrowth. Instead of a single response by duct outgrowth, there is a triple response to the one hormone. It is like a pseudo-pregnancy response. It is pathological in that it is irregular, distorted by distension, and patchy in distribution within the glands. In extent it did not exceed the

limits of outgrowth attained during normal pregnancy. It is pathological also in that the degree of differentiation is suggestive of a 9th–12th day pregnancy while the milky secretion is voluminous. Male glands reacted to larger doses in a similar manner. They were often more irregular in arrangement of ducts and acini. In normal stock mice and in rats also the response of the mammae to spontaneously secreted or to injected oestrogens is by simple duct outgrowth (Fig. 7). Lobular-alveolar growth requires the stimulus of pregnancy or of artificially applied progesterone in addition to oestrogen (Mixner and Turner, 1943). Normal unmated female mice have no luteal phase in the ovarian cycle and are said to secrete no luteal hormone. The atypical oestrogen response of R3X and Simpson mice is a hormone conditioned hypertrophy and hyperplasia, including adenosis and secretion by the glandular cells. That it is atypical, even among inbred strains, is shown by comparison with C57 (black) and Strong AX virgin female mammae after identical treatment. No dose of oestrogen was found that gave a similar response in C57 (black) females or males or in Strong A or AX females or males. Continued treatment over a long period of time sometimes resulted in slight local acinus formation in Strong A mice. At the age of one month no response was observed in R3X females save for a tendency of the end bulbs to canalize.

The minimum dose of oestrogen required to reveal this capacity for atypical responses in R3X mice and the time after treatment when it first appears have not yet been found. This atypical response is the cystic disease of mouse mammae first described by Haaland (1911) as occurring spontaneously in mixed stock (though in these animals a milk-borne agent may also have been present), and later by Gibson (1930) in inbred strains. It was first reproduced experimentally in mixed stock mice in 9 out of 11 spayed females by Goormaghtigh and Amerlink (1930), who compared it with Reclus disease in the human breast. Experiments here recorded provide the additional information that the response can be provoked independently of the presence of milk-borne tumour agent, and confirm opinions previously expressed that it is a strain characteristic. It is of interest that in a small series of virgin and breeding R3X females cystic distension has never been observed to occur spontaneously (in 25 out of 25 examined).

The atypical response might be due either to local differences in sensitivity of the end bulbs of various strains of mice or to the response in the pituitary gland, where, according to Gomez and Turner (1937; Gomez, Turner and Reece, 1937) the effective mammogenic hormones are produced, or to a combined response in both organs. Some light on this problem was gained from experiments similar to those here described using the double dose of 400 microgrammes of oestradiol on spayed F.1 hybrid females of the C57 x R3X cross. The curious observation was made that a similar lobular-alveolar response was provoked in all the mammae, but it was localized in approximately half of each gland. The other half responded typically like the C57 (black) parent's gland (Fig. 8). These results suggest that the atypical response is due in part at least to variation in local sensitivity of ducts and end bulbs, and not solely to a strain difference in the pituitary response.

In the absence of ovaries and of any further application of hormone the characteristic but atypical reaction persisted in R3X mice for about ten weeks after the beginning of the experiment, using 800 microgrammes of oestradiol.

Regression of the whole of the recent acinar and pseudo-lobular-alveolar development started after about the 10th week, and was complete in most of the mice between the 16th and 20th week. The mice were then 6 to 7 months old. The exact time taken for complete regression varied with the degree of the original response, which in its turn varied slightly in individual mice. Complete regression was indicated by total disappearance of all lobules, acini and secretion. Bare, shrunken, almost linear ducts remained (Fig. 5). Details of the number of mice examined in this way and of complete or incomplete regression of the hormone response are given in Table I. Some of the animals killed at 16 weeks revealed remnants of atrophic pigmented acini usually in small groups at the periphery of the 4th mammae, probably at the site of former large clusters. Fig. 9 is representative of these imperfections of involution. Sometimes detached ghost acini remained unabsorbed (Fig. 10). No inflammatory or metaplastic nodules were encountered.

In the course of these experiments the possibility had to be considered that in the females some other sources of oestrogens might be stimulated by ovariectomy and after cessation of the action of artificially applied hormone to produce oestrogens, as Smith and Bittner (1945) found in the C3H strain. That no such stimulation had occurred was proved by making vaginal smears during the last two to three weeks of the experimental period in 11 out of 20 mice, and by inspection of uterus and oviducts at autopsy. In no case was there a return of oestrus or failure of uterus and oviducts to atrophy.

R3 Virgin Females Deprived of their Ovaries but Containing the Milk-borne Tumour Agent.

Early changes in the mammae in response to ovariectomy and oestrogen treatment (ketohydroxyoestrin and oestradiol) were similar to those described in the same strain deprived of the tumour agent (32 out of 32 mice). Differences were detectable in the intervening period, but it will be convenient first to contrast the final results at 16 to 20 weeks in the two sets of mice. Proliferation due to hormone action regressed at a similar rate and was complete, so far as this can be judged in the presence of the tumour agent, at some time between the 16th and 20th weeks of experiment. Regression was expressed in the greater part of all mammae by disappearance of lobules and alveoli and by the presence of a shrunken duct system. In the majority of mammae, however, there persisted localized nodular areas of proliferation of acini on the duct system (Fig. 6). These were found in all of 28 mice and, in addition, 11 out of 40 mice killed before the 8th month of life bore mammary adenocarcinomas. The earliest of these appeared at four-and-a-half months old. The nodules of proliferation occurred on otherwise atrophic duct systems, giving a picture similar to that described by Gardner *et al.* (1939). By the vaginal smear technique, oestrus cells were seen on one occasion in each of 2 mice out of 32 examined. In 30 mice oestrus cycles or cells did not recur.

The contrast between the mammae in the two sets of mice at 16 to 20 weeks was striking. There was complete absence of adenomas in those mice which had been deprived of the milk-borne tumour agent, whereas in mice of the original R3 strains carrying milk agent adenomas were numerous in nearly all the glands of every mouse. Fig. 5 and 6 are representative of 200 mammae

from the R3X females and 280 from those carrying the agent. A few imperfections due to incomplete regression did not cause confusion, and will be referred to in this connection again.

The conclusions were drawn that the foci of hyperplasia (adenomas) in the R3 females carrying tumour agent, were due in respect of their origin and persistence to the presence of a milk-borne tumour agent, presumably identical with or a variant of Bittner's agent, and that this agent had been forced into activity by the large dose of hormone. These nodules are considered to be experimentally produced counterparts of those that occur spontaneously in all R3 females after the age of 8 to 9 months. They are similar to those described in great detail, and finely illustrated by Gardner (1942) in other strains. Gardner demonstrated the persistence of spontaneous pregnancy-activated adenomas in the absence of further hormone stimulation in hypophysectomized breeder females. The present experiments provide a simple method of forcibly stimulating adenomas and of revealing their dependence on the milk-borne tumour agent.

A few more descriptive details that are characteristic of R3 adenomas may be added to Gardner's comprehensive accounts. Both spontaneous and forcibly activated R3 adenomas are of two main varieties, compact (Fig. 6) and diffuse (Fig. 11) clusters of acini connected by shorter or longer lengths of duct. Some of the former appear to lack duct formation altogether. Some of the latter may be largely composed of ducts, similar to one of Gardner's, or even of end-bulbs. Many of the diffuse loosely constructed adenomas contain secondary foci of more active proliferation (Fig. 11 and 12), indicating perhaps sites of greater vigour of the stimulating agent, and providing evidence for the existence previously suggested of variants of the agent. These secondary foci were found frequently in both spontaneous and artificially activated adenomas, including many in males treated with hormone. They too were independent of ovarian hormone for maintenance once they had been activated. No inflammatory or metaplastic nodules were encountered.

The earliest date at which adenomatous proliferation was found was at 21 days after a single dose of 200 microgrammes of ketohydroxyoestrin (Fig. 14). This finding was not constant at 21 days. Adenomas were found in 4 out of 4 mice at 9 weeks after 800 microgrammes given in 2 doses of 400 microgrammes each at 20-day intervals, applied to spayed females at approximately 56 days old. At these dates the hormonal response in the mammae was still in existence, and the interpretation of an adenomatous response was open to question (Fig. 13). The differentiation between focal hyperplasia or adenosis caused and maintained by hormone alone and adenomatous nodules caused and maintained by milk-borne tumour agent plus hormone is possible in some nodules only. In others no certain decision can be reached. The chief difference in the two kinds of clusters of acini rests on their spatial arrangement and relative regularity. The hormone induced hyperplasias tend to lie in one plane ; the combined response of hormone and milk-borne tumour agent is exceedingly irregular in comparison and lies in all dimensions (Fig. 3 and 13). When, as in one adenomatous nodule seen at 3 weeks (Fig. 14) numerous end bulbs were observed arising from a cluster of acini, there can be no doubt of the pathological and atypical origin of the proliferation due to stimulation other than by hormones. End bulbs, though sometimes multiple, are normally situated at the tips only of growing ducts

(Cole, 1933). This is also the case when artificially supplied hormones are acting alone.

The smallest total dose of oestrogen which constantly activated the milk-borne tumour agent into pathological activity as shown by adenoma production was 50 microgrammes of ketohydroxyoestrin given through the skin in a small series of mice (Table I).

R3X Males Deprived of the Milk-borne Tumour Agent.

The rudiments of mammae of males derived from the same sublines as the females and treated with larger doses of ovarian hormones responded in a similar manner. An average of 6 mammary glands per mouse developed from rudiments and all examined revealed the atypical response characteristic of females of this strain. Irregular lobules were seen after cutaneous application of 800–1200 microgrammes of oestradiol with or without 1500 microgrammes of progesterone. Regression required 6 to 7 months to become complete, leaving bare atrophic ducts (Table II).

R3 Males Containing Milk-borne Tumour Agent.

When additional time had been allowed for regression of hormone response, results were similar, and almost as regular as in spayed females containing milk-borne tumour agent. Either oestrogen alone (1200 microgrammes) or combined with one dose of progesterone (1,500 microgrammes) was sufficient to stimulate the growth of adenomas (Table II). Adenomas were found in 38 out of 40 male mice, but were not so numerous in individuals as in the females. Nine of the males developed malignant tumours by this treatment in 5 months.

Twelve males which were castrated before treatment with hormone developed adenomas but no malignant tumours. The hormone dosage used was better tolerated by all the male mice than by the more usual protracted method. Results were not better in respect of stimulation when progesterone was added than in experiments in which oestradiol alone was used (Table II) but a larger number of malignant growths was seen.

Other Varieties of Mice.

The same methods were applied in preliminary tests to C3H, Strong A, Simpson and C57 (black) strains. Results are summarized in Table III. The fact that mammae of Strong A females respond normally to oestrogen, that is by duct outgrowth only, indicates that the atypical pseudo-lobular-alveolar response probably does not represent a structural basis of the hereditary factor underlying susceptibility to pathological activity of milk-borne tumour agent.

Since it might be suggested that the lobular-alveolar response is an expression of a mutation in some highly inbred strains, a few stock mice obtained from a dealer were examined by the same technique as for hormone response. These were got from a town where, so far as one knew, no inbreeding of pure lines was being carried on. This precaution was taken to avoid mice that originated from pure line laboratory bred strains. One out of 11 of these mongrel mice responded with early widespread pseudo-lobular-alveolar differentiation and secretion. In degree the distension and proliferation were less than in some

of the inbred strains, but in distribution and kind the reactions were very similar. This indicates that if the reaction is based on a mutation, then the mutation can occur also under relatively natural conditions.

The test for activation of milk-borne tumour agent has been applied to reciprocal hybrids and the results are incomplete. They indicate so far that hybrids are suitable experimental material for the present purpose (Table III).

COMMENT.

The work here recorded confirms the opinion already widely held that hyperplastic nodules in the mammae of mice provide evidence of pathological activity of the milk-borne tumour agent, and shows how this activity can be forced and detected sooner than by the spontaneous appearance of mammary tumours. The presumption is that the agent provoking nodular hyperplasia and the tumour agent are the same or of like nature, because in both cases the response is by cell proliferation. Further work is required to settle the degree of similarity. The two objections to accepting nodular hyperplasia as evidence of activity of the agent have been met. The criteria proposed as bases for the validity of the test were twofold. They required the absence of nodular hyperplasia (adenomas), and of all other signs of acinus formation in the absence of the agent and absence of hormone. They required the development of adenomas in involuted mammae in the presence of the agent and absence of hormone. As has been recorded, perfection in respect of complete absence of acini required in the first criterion was attained in 15 out of 20 females; in 5 others killed at an earlier date acini were found which were regarded as being, at the time of death of the mice, in process of regression. There was, in the authors' opinion, no reasonable possibility of confusing these surviving acini with adenomas. The surviving acini were grouped along the periphery of the gland (Fig. 9) in a manner bearing no resemblance to adenomas (Fig. 6). Their shrunken atrophic character was apparent. It is probable that the provocative doses of hormone were larger than needed for the purpose, and that given the same time for involution to take place a smaller dose would have been succeeded by complete disappearance of acini. Alternatively a longer interval must be allowed for the larger dose. Regression of hormone response was more often complete in males, probably owing to the longer time interval allowed for it (Table II). Success in respect of the second criterion was attained in 28 out of 28 females and in 28 out of 30 males.

SUMMARY.

1. Variations in response to oestrogenic hormones in the mammae of spayed females of several strains of mice have been confirmed. They have also been found in mice from a dealer. The variations consist of pseudo-lobular-alveolar differentiation and secretion of milky fluid in addition to the normal response of duct outgrowth. The response is a pathological hyperplasia or adenosis of the mammary gland. It is independent of the presence of the milk-borne tumour agent. In the absence of further oestrogenic stimulation in spayed mice this pseudo-lobular-alveolar response regressed, leaving bare shrunken ducts.

2. A second type of response was elicited in R3 and C3H mice carrying the milk-borne tumour agent. This second response was identified with certainty as

caused by the milk-borne tumour agent (Bittner's or a variant of it) only when all other formative agents were excluded. This was done by spaying and providing a limited dose of hormone to provoke pathogenic activity of the agent. It was characterized by the appearance of adenomas in otherwise involuted mammae. It is suggested that these adenomas are to be regarded as evidence of activity of milk-borne tumour agent when all other formative agents have been excluded, whether they occur spontaneously or are forcibly stimulated.

REFERENCES.

ANDERVONT, H.—(1945) *In* a Symposium on Mammary Tumours in Mice. Washington, D.C., p. 134.

APOLANT, H.—(1906) *Arb. a. d. Königlichen Inst. exp. Therap., Frankfürt A.M.*, **1**, 7.

BITTNER, J. J.—(1936) *Science*, **84**, 162.—(1939) *Publ. Hlth. Rpts.*, **5**, 1590.—(1943) *Cancer Res.*, **3**, 441.—(1945) *In* the A.A.A.S. Research Conference on Cancer. Washington, D.C., p. 63.

Idem, HUESBY, R. A., VISSCHER, M. B., BALL, Z. B., AND SMITH, F.—(1944) *Science*, **99**, 83.

BONSER, G. M.—(1936) *J. Path. Bact.* **42**, 169.

BURROWS, H.—(1936) *Ibid.*, **42**, 161.—(1945) 'Biological Actions of Sex Hormones,' Cambridge, p. 330.

COEN, E.—(1887–88) *Beitr. Z. path. Anat. u. Physiol.*, **2**, 85.

COLE, H. A.—(1933) *Proc. Roy. Soc.*, B., **114**, 136.

GARDNER, W. U.—(1942) *Cancer Res.*, **2**, 476.

Idem, DIDDLE, A. W., ALLEN, E., AND STRONG, L. C.—(1934) *Anat. Rec.*, **60**, 457.

Idem, SMITH, G. M., AND STRONG, L. C.—(1935) *Proc. Soc. exp. Biol. Med.*, **33**, 148.

Idem, STRONG, L. C., AND SMITH, G. M.—(1939) *Amer. J. Cancer*, **37**, 510.

GIBSON, L. M.—(1930) *J. Cancer Res.*, **14**, 570.

GOMEZ, E. T., AND TURNER, C. W.—(1937) *Bull. Mo. agric. Exp. Sta.*, No. 259.

Iidem AND REECE, R. P.—(1937) *Proc. Soc. exp. Biol. Med.*, **36**, 286.

GOORMAGHTIGH, N., AND AMERLINK, A.—(1930) *Etud. Cancer*, **19**, 527.

v. GULIK, P. J., AND KORTEWEG, R.—(1940) *Nederl. Akad. v. Wetensch.*, **43**, 891.

HAALAND, M.—(1911) 'Fourth Scientific Rep. Imp. Cancer Res. Fund, London,' p. 1.

HUESBY, R. A., AND BITTNER, J. J.—(1946) *Cancer Res.*, **6**, 240.

KIRSCHBAUM, W., WILLIAMS, W. L., AND BITTNER, J. J.—(1946) *Ibid.*, **6**, 354.

LACASSAGNE, A.—(1934) *C.R. Soc. Biol., Paris*, **115**, 937.

LATHROP, A. E. C., AND LOEB, L.—(1916) *J. Cancer Res.*, **1**, 1.

MACKENZIE, I., AND ROUS, P.—(1941) *J. exp. Med.*, **73**, 391.

MIXNER, J. P., AND TURNER, C. W.—(1943) *Bull. Mo. Agric. Exp. Sta.*, No. 378, p. 20.

ROUS, P., AND KIDD, J. G.—(1941) *J. exp. Med.*, **73**, 365.

SHIMKIN, M. B.—(1945) *In* a Symposium on Mammary Cancer in Mice. Washington, D.C., p. 97.

SMITH, F. W., AND BITTNER, J. J.—(1945) *Cancer Res.*, **5**, 588.

TURNER, C. W., AND GOMEZ, E. T.—(1933) *Bull. Mo. agric. Exp. Sta.*, No. 182.—(1934) *Ibid.*, No. 206.

WOGLOM, W. H.—(1945) *Cancer Res.*, **5**, 576.

ARSENICAL CANCER: A REVIEW.

O. NEUBAUER.

Published for the Arsenic Committee of the Medical Research Council.

Received for publication June 15, 1947.

TABLE OF CONTENTS.

A. Medicinal Arsenical Cancer.

I. Historical.
II. Diseases for which Arsenical Drugs were taken.
III. Sex of the Patients.
IV. Arsenical Drugs Used.
V. Duration of Administration of Arsenic.
VI. Quantity of Arsenic Taken.
VII. Symptoms of Arsenicism Preceding and Accompanying Arsenical Cancer.
VIII. Clinical Pictures of Medicinal Arsenical Cancer.
1. Arsenical Epithelioma of the Skin Developing in Keratotic Areas (Hutchinson Type or Keratoseogenic Cancer).
2. Arsenical Epithelioma Arising in a Patch of Psoriasis.
3. Arsenical Epitheliomas in Normal Skin.
4. Multiple (Benign) Superficial Epitheliomata.
5. Bowen's Disease.
6. Extramammillary Paget's Disease.
7. Arsenical Cancer in other Organs than the Skin.
8. Combination of Polymorphous Types.
9. Frequency of the Different Types of Medicinal Arsenical Cancer.
10. Multiplicity of Medicinal Arsenical Cancer.
11. Distribution of Medicinal Arsenical Cancer.
12. Chronological Course of Medicinal Arsenical Cancer.
13. Age at Onset of Medicinal Arsenical Cancer.
IX. Histology of Arsenical Cancer.
X. Chemistry and Histochemistry in Arsenical Cancer.
XI. Prognosis of Arsenical Cancer.
XII. Treatment of Medicinal Arsenical Cancer.

B. Arsenical Cancer Caused by Drinking Water.

Reichenstein Disease—Cordoba Disease—Other Occurrences.

C. Occupational Arsenical Cancer.

I. Arsenical Cancer in Miners and Smelters.
II. Arsenical Cancer in Factories.
1. Sheep Dip Factories.
2. Cancer Caused by Arsenical Insecticides.
3. Arsenical Cancer Caused by Coal and its By-Products.
4. Cancer of the Bladder in Aniline Workers.
III. Comment on Occupational Arsenical Cancer.

D. Arsenical Cancer in Animals (except Experimental Cancer).

Cornwall and Wales—Freiberg—Tharandt Forest—Limbach.

E. Experimental Arsenical Cancer.

Local Application on the Skin.
Oral Application of Arsenic.
Parenteral Application of Arsenicals.
Influence of Arsenic on Pre-existing Cancer.
Combination of Arsenic with other Agents (Carrel—Askanazy—Fischer-Wasels).

F. Pathogenesis of Arsenical Cancer.

Relations to Keratoses and Pigmentation—Local Effects of Arsenic—General Effects—Action on Capillaries—Action on Metabolism—Stimulation of the Cells—Predilection for the Skin—Prolonged Latent Period—Individual Reactivity—Heredity—Embryonic Germs—Co-operation of Several Factors.

References.
Tables.

A. MEDICINAL ARSENICAL CANCER.

I. Historical.

THE first observer to direct general attention to the role of arsenical drugs as an etiological factor in cancer of the skin was Sir Jonathan Hutchinson.* He described in 1887/88 five cases leading him to the idea that the internal administration of arsenic in large doses over long periods might produce a form of cancer which presents certain peculiarities. In all cases a condition of local keratosis had preceded the cancer ; in one case the cancerous ulcer had developed on rough skin on the side of the trunk, in one case on a corn on the scrotum, whilst in all the others it occurred in keratotic areas on the hands or feet.

In the following years the views of Hutchinson were discussed. An always rising number of similar cases was reported. Hartzell (1899) was able to collect 10 cases ; Wile (1912) 19 cases ; Pye-Smith (1913) 31 cases, 24 of them of apparently medicinal origin. Nearly all authors (Hartzell, 1899; Ullmann, 1900; Schamberg, 1907; Dubreuilh, 1910; Pye-Smith, 1913; Bland-Sutton, 1916; Wise, 1920) agreed with the teaching of Hutchinson. Wile (1912) made an " impartial statement " of the facts for and against the specificity of arsenical cancer. Only a few authors, as de Silva (1918), opposed the views of Hutchinson ; de Silva admitted that arsenic is responsible for the production of the keratotic condition, but regarded the development of cancer as being independent of the drug. Eventually the views of Hutchinson were accepted by all dermatologists.

Then the predominance of a history of psoriasis in cases of arsenical cancer was especially discussed (see p. 194).

A new period in the history of medicinal cancer started with the establishment and characterization of the clinical picture called multiple superficial epitheliomatosis, and the discussion of the etiological role of arsenic in this condition. These discussions mainly in the dermatological societies since about 1930 have not yet led to general agreement (see p. 201). The same is true

* Preceding the appearance of Hutchinson's report several authors had already described the occurrence of cancer of the skin in cases of psoriasis (Pozzi, 1874 ; Tillaux, 1877 ; Cartaz, 1878 ; White, 1885 ; and Hebra, 1887 ; *cit.* Pye-Smith, 1913*b*) ; but they had not associated the development of cancer in these cases with the previous administration of arsenicals for treatment.

concerning the condition called Bowen's disease and extramammillary Paget's disease and their relations to ingestion of arsenic. It was tried to clear these questions by the application of chemical and especially histochemical methods of investigation for arsenic in the growths and in the unaffected skin of the patients.

In Table I are collected 143 published cases of medicinal arsenical epithelioma in man. Not included are :

(*a*) About 13 cases in which neither the original paper nor a reliable abstract could be obtained : Toeroek, 1891 ; Crocker-Hartzell, *cit.* Hartzell, 1907 ; Foenss, 1921, 1923 (two cases ?) ; Schaumann, 1922, 1923 ; Papagaaw, 1926 ; Urbach, 1926 ; Ullmann, 1930, 1933 ; Mayer, 1933 ; Schaerrer, 1934 ; Simons, 1936/37 ; Zieler, 1938, *cit.* Voss, 1939 ; Koerbler, 1939 ; Rille, *cit.* Voss, 1939 ; Hauser and Simon, 1941.

(*b*) Cases published without detail : Spencer, 1913 ; Oliver and Finnerud, 1928, " case 2 " ; Montgomery, 1935 (about 11 cases) ; Rosen, discussion to Montgomery, 1935 (several cases) ; Cannon, discussion to Montgomery, 1935 ; Nomland, *cit.* Waugh and Scull, 1935 (two cases) ; Anderson, discussion to Montgomery and Waisman, 1941 (a number of cases) ; Gammel, discussion to Barney, 1944 ; Ellis, discussion to Anderson and Burpeau, 1945 (two cases) ; Roxburgh, discussion to Russell and Klaber, 1945.

(*c*) About 15 cases in which the administration of arsenicals is either doubtful or not mentioned at all, but may be assumed as probable : Pozzi, 1874 ; Tillaux, 1877 ; Cartaz, 1878 ; Whitfield, 1906 ; Sargent, 1906 ; Wende, 1908 ; Remenovsky, 1921 ; Ketron, 1927 (two cases) ; Dreyer, 1927, *cit.* Buergener, 1939 ; Stillians, 1930, *cit.* Anderson, 1932 ; Cheever, 1931, *cit.* Buergener, 1939 ; Franseen and Taylor, 1934 (5 cases, " cases 11–15 ") ; Guszman, 1934, *cit.* Buergener, 1939 ; Opfer, 1935, *cit.* Buergener, 1939 ; Montgomery and Waisman, 1941, " case 3."

(*d*) Four cases in which the diagnosis cancer (epithelioma) is not certain : Schamberg, 1907, " case 2 " ; Sawyer, 1929 ; Lane, 1939 ; Abramowitz, Mattice and Bottvinck, 1944, " case 8."

(*e*) Seven cases in which besides arsenic other potential carcinogenic agents (X-rays, radium) were used : Schwartz and Busman, 1924 (X-rays) ; Mayer, 1927, *cit.* Buergener, 1939 (X-rays) ; Stillians, 1931 ; Blumenthal, 1931, *cit.* Buergener, 1939 (X-rays) ; Milch, 1932 (radium) ; Glen, 1945 (radon) ; Anderson and Burpeau, 1945 (X-rays).

II. Diseases for which Arsenical Drugs were taken.

Considering the vast number of conditions in which arsenic is used for long periods, the great percentage of cases in which arsenical cancer has developed in patients suffering from skin-diseases (in 89 = 71·2 per cent), and especially from psoriasis (in 67 = 53·6 per cent), of all cases with noted diagnosis, is very impressive. It seems to be more than a mere coincidence. Hartzell, 1899; Wile, 1912 ; Alexander, 1921 ; Remenovski, 1921 ; Templeton, discussion to Alderson (1935) ; and Buergener, 1939, have discussed this question (see p. 200). On the other hand the number of cases developed in arsenic-treated cases of different internal disorders is relatively small ; surely some of these diseases may have

Out of the 143 cases in Table I, the disease for which arsenic has been administered is mentioned in 125 cases:

For Psoriasis	67 cases	53·6 per cent	
Pemphigus	3		
Eczema, Dermatitis	4		
Dermatitis herpetiformis	4		
Acne	6	22 cases, 17·6	,,
Lichen ruber	1		
Pruritus	1		
Unknown disease of skin	3		
Sum of cutaneous diseases	89 cases.	71·2	
Anaemia and haemorrhagic diathesis	4 cases.		
Asthma and chronic bronchitis	6 ,,		
"Stomach trouble"	1 ,,		
Epilepsy and "fits"	11 ,,		
Chorea	3 ,,		
Ataxia (?)	1 ,,		
Nervousness	3 ,,		
As "tonic" and for "complexion"	4 ,,		
Malaria	1 ,,		
"Syphilis"	2 ,,		
Sum of diseases without diseases of the skin	36 ,,	28·8	,,
Diseases not known in	17 ,,		
		100	,,

ended with death before an arsenical cancer had time to develop as in cases of pernicious anaemia, leukaemia, and Hodgkin's disease; but this explanation would not be valid for the less dangerous forms of anaemia, for asthma and the great number of patients with asthenia in which prolonged and repeated medication with arsenic is or was in common use. And the appearance of arsenical cancer as a sequel to treatment of syphilis with arsenicals is really a rarity. Only 2 or perhaps 3 cases (50, 53, 108 ?) are contained in Table I. Besides, Cannon (discussion to Montgomery, 1935) mentioned several patients with multiple basal-celled epitheliomas who had received injections of arsphenamine for syphilis, but no details were given. Spencer (discussion to Pye Smith, 1913*a*) mentions another case.

So it seems that affections of the skin, and especially psoriasis, give a predisposition for arsenical cancer, although there is no real definite proof.

III. Sex of the Patients.

In 131 cases of Table I the sex is noted; 91 were males and 40 females; that means a sex relation of 2·3 : 1. This predominance of males is not a

characteristic of arsenical cutaneous cancer, as it is found in cancer of the skin in general.

IV. Arsenical Drugs Used.

In nearly all cases *inorganic drugs* were used, containing arsenic in its *trivalent form*; that means derivatives of arsenious acid, the most common drug being potassium arsenite $KAsO_2$; it was mostly given in aqueous solution (1 per cent calculated as As_2O_3 = Fowler's solution), or as arsenious anhydride = arsenic trioxide As_2O_3 in form of the so-called asiatic pills = black pills (each containing usually $\frac{1}{12}$ gr.), or as Donovan's solution (containing 1 per cent AsI_3 plus 1 per cent HgI_2. In two cases "Ascato" was taken, a patent medicine for the treatment of asthma; in one case "Dr. Greene's Nervinum." Nearly always the drugs were ingested by mouth; in two cases they were also injected.

Pentavalent inorganic compounds—derivatives of arsenic acid, H_3AsO_4, are not in use as medicaments; so it is not surprising that no case is known in which arsenical medicinal cancer has been observed on this basis; but, as it is known that pentavalent arsenicals are reduced in the body into trivalent ones (see p. 230), it can be understood that they play a role in occupational cancer (see p. 217).

Some confusion has arisen in the literature of arsenical cancer about the denominations "trivalent" and "pentavalent." It seems to be a mistake that Osborne (1925, 1928) quoted As in arsenious acid as "quintavalent" (pentavalent), and some authors (Milch, 1932; McNeer, 1934, and Montgomery, 1935) followed him. Generally arsenious acid is considered as trivalent, although some reasons may be produced for its being pentavalent in the vitreous arsenious anhydride (Erdmann, 1904).

The most important *trivalent organic* arsenical compounds, the arsphenamins so generally used in the treatment of syphilis, play only an insignificant role in the causation of cancer. It is true that arsenical keratoses after their administration have been observed repeatedly (MacLeod, 1913; Gauvin, 1927; and Timberlake, 1929). Spencer, discussion to Pye-Smith (1913*a*), was told of a case of arsenical epithelioma traceable to salvarsan, and Galloway (*ibid.*,) and Ullmann (1917) have sounded warnings that more incidences might be expected. In the case of Harbitz, 1927 (case 53), after injections of arsphenamine a malignant tumour developed at the site of the injection, a fibrosarcoma, different from the usual kind of arsenical cancer. Rosen (discussion to Montgomery, 1935) has seen several cases in which the treatment of syphilitic affections of the mouth with arsphenamine had led to the development of epitheliomas of the oral mucosa. Cannon (*ibid.*, 1935) has observed basal cell epitheliomas in several patients who had received many injections of arsphenamine a number of years before. Besides, arsphenamines were administered in the cases of Levin (1926) (case 50) and of Milch (1932), but in combination with trivalent inorganic arsenicals. For Ebert's (1929) results with intradermal application of arsphenamine see p. 226, Experimental Arsenical Cancer.

Some *pentavalent organic* arsenicals as cacodylates, atoxyl and arsacetine are—or were—frequently used as medicaments; but it seems that they never have proved as carcinogens; only in case 135 possibly cacodylates were used.

No case in known in which *external* application of arsenic as medicament (as a caustic, e.g. in lupus, or in the odontologic practice) has produced a cancer.

V. Duration of Administration of Arsenic.

Duration of exposure to arsenic in cases of medicinal arsenical cancer is reported only in a certain number of the cases. Among 96 cases of Table I the distribution was as follows :

6 weeks	3 cases.
2 months	1 case.
6 ,,	4 cases.
Up to 1 year	3 ,,
1–5 years	22 ,,
6–10 years	13 ,,
11–20 ,,	16 ,,
21–30 ,,	8 ,,
More than 30 years	4 ,,
" A short time "	2 ,,
" A considerable time "	1 case.
" From time to time "	1 ,,
" Several years "	4 cases.
" Many years " or " long time "	16 ,,
	98 ,,

In the great majority cancer resulted only after the drug had been administered for a very long period, up to 36 years (case 38). But there are also cases in which a very short time of exposure was sufficient for the later development of malignancy (cases 37, 79, 91, 123 and 128).

VI. Quantity of Arsenic Taken.

Similar great differences can be noted in comparing the doses of arsenic leading to the development of malignancy. Only in about 16 of the patients in Table I the doses and the time of administration are sufficiently noted ; in one case (case 83) a quantity of only 0·19 g. As_2O_3 was given, whereas case 23 received in all about 70 g., and case 124 about 121 g. The average quantity was about 28 g.—about 430 grains.*

VII. Symptoms of Arsenicism Preceding and Accompanying Arsenical Cancer.

The development of arsenical cancer is frequently—often for many years—preceded by non-malignant manifestations of arsenicism. Some of these seem to be without any direct relation to the origin of malignancy, whereas others, especially the keratoses, often give rise to the appearance of cancer, and have, therefore, to be regarded as really " precancerous " manifestations.

Only rarely signs of acute arsenical poisoning are mentioned in the history ; and even certain usual signs of chronic arsenicism, as neuritis, anaemia, nephritis and affection of the liver, have been observed in only a few of the patients with arsenical cancer.

* It is supposed that Fowler's solution, given in the great majority of cases, was always undiluted (1 per cent As_2O_3).

A sign of chronic arsenicism in those patients, often but not constantly reported, is *hyperpigmentation* (melanosis) of the skin. The patients exhibit a brownish-yellow tint usually most marked in places which normally are more pigmented (areolae of the nipples, about the axilla, in the groins, on the perineum, etc.), and in sites of pressure (waist of women, beneath the garter, etc.), and on previous lesions of the skin. The pigmentation occurs in patches, often forming reticular mottling, or showing a " rain-drop "-like appearance. The pigmentation is not unlike that in Addison's disease ; but in this condition the affected skin is not so harsh and scaly, and often the mucous membranes are affected too (in arsenical melanosis only exceptionally).

In 25 of the cases in Table I hyperpigmentation is noted ; but there may be many more in which it was present. Only in 11 cases the absence of pigmentation is definitely stressed.

Other changes of the skin reported are : telangiectases (cases 20, 85, 117 and 124), chronic arsenical dermatitis (case 48), erythrodermia (case 47), erythrodermia exfoliativa (cases 51, 69 and 70), multiple seborrhoeic warts and molluscum fibrosum (cases 140 and 141), seborrhoeic keratoses (cases 74 and 75), and affections of the nails (see Willcox, discussion to Hamilton, 1921).

But by far the most important manifestations preceding the appearance of arsenical cancer are *keratoses*. Henoch and Romberg in 1851 (Geyer, 1898) have recognized epidermic desquamation of palms and soles as a sequel to the internal use of arsenic, and in 1868 Erasmus Wilson has given a specified description of this condition. Hutchinson (1887) has emphasized their importance as precursors of arsenical cancer. (Pictures, see Hutchinson, 1888, plates xxi and xxii, 1895, plates xvii and xix.)

These keratoses occur especially on the palms and soles, but may be found anywhere on the cutaneous surface. The most characteristic form, the nodular form (punctate keratosis) of the palms and soles, is marked by numerous small horny corn-like elevations usually 2–5 mm. in diameter. They occur frequently as epidermal pegs which can be picked out of their keratotic beds. They are frequently situated on the thenar and lateral borders of the hand, on the roots or on the side surfaces of the fingers, sometimes also on the back of the phalangeal joints. On the feet the sites of predilection are the heels and the toes. There may be a confluence of a group of these corns forming wart-like excrescences, but differing from true warts by having no papillary structure. Sometimes the palms only or the soles only are affected. In other cases there is a more diffuse keratosis of the palms and soles, giving the skin a leathery appearance, often accompanied with or preceded by hyperidrosis. The horny thickenings may be situated on an erythematous base, or a horny patch may be surrounded by an erythematous halo. Frequently the diffuse form of keratosis is combined with the nodular form.

The keratoses of palms and soles are usually symmetrically distributed. Fissures are liable to occur on the keratoses.

Slight keratoses may also occur on other parts of the body, rendering the skin harsh, or flat reddish erythematous patches, or scaling keratotic plaques may be found. They occur mostly on the unexposed parts of the body, in contradistinction to the keratoses of senile and of weather-beaten persons.

These keratoses are present in the great majority of cases of arsenical cancer. They are noted in 116 out of the 143 cases of Table I. In only 10 cases their

presence is denied ; all these are apparently cases of superficial epitheliomas (see below), one perhaps a true psoriasis cancer (case 24, see below). In the remaining 18 cases nothing is said about keratoses, the histories often being short and deficient. So it can be stated that keratoses occur in from 81 to 93 per cent of all cases of arsenical cancer. Hands or feet or both were involved in at least 97 cases, mostly in the classic form of corns on palms and soles, frequently combined with keratoses on other parts of the body. In only two or three cases keratoses were present but spared hands and feet. In case 74 a keratosis appeared in the form of a cutaneous horn in the right groin ; in case 47 a distinct hyperkeratosis subungualis is noted. Leukoplakia oris was present in cases 33 and 129.

The keratoses develop gradually, and may not appear for some time after the ingestion of arsenic. The interval between the beginning of exposure to the poison and the appearance of keratotic lesions is very different in different cases. (See p. 210, Chronological Course.)

Many of the keratotic areas become stationary, or may even undergo a gradual involution after the use of the drug has been stopped (Montgomery, 1935). But some of them develop progressive changes—painful fissures, infections, etc.—and eventually may give rise to malignant growth ; in some cases the malignancies originate on other parts of the body (see below). Montgomery (1935) states that out of his 85 cases of arsenical keratosis 20 per cent developed epitheliomas.

The multiple horny keratoses, especially the punctate or warty form on the palms and soles, are generally recognized as being pathognomonic for chronic arsenical poisoning ; they can be confounded only with certain hereditary forms of keratosis punctata. Nearly all dermatologists agree that their mere presence justifies the diagnosis of arsenicism even in cases lacking any history of ingestion of the poison (see Franseen and Taylor, 1934 ; Oliver, discussion to G. Lane, 1939 ; and Montgomery and Waisman, 1941, " case 3 "). Hence their great importance for the diagnosis—as real " leading fossils " for prophylactic therapy and for the consideration of the pathogenesis.

VIII. Clinical Pictures of Medicinal Arsenical Cancer.

The development and appearance of arsenical growths are by no means the same in all cases. There are several types differing in localization, in the grade of malignancy, in the histological picture, etc.

1. *Arsenical epithelioma of the skin developing in keratotic areas (Hutchinson type or keratoseogenic cancer).*

These cases, described in such a classic manner by J. Hutchinson, form a most characteristic group. After the keratotic lesions, especially those of the hands and feet, have existed for a long time, sometimes for years, one of them—or some of them—begin to undergo certain changes : they become painful, bleeding fissures appear, and after a certain time, sometimes after years, an ulcer appears with hard pearly borders slowly extending without tendency for healing. Histological examination shows an epithelioma usually of the squamous cell type. Sites of predilection are the hands, especially the fingers, palmar and lateral surfaces, thumb, the borders of the palms, the heel and toes, but any

arsenical keratosis may give rise to such a malignant degeneration. At the same time or later another malignant ulcer may appear on a keratosis in the vicinity or elsewhere, or even in a non-keratotic area; and then another and so on.* If not treated in time, earlier or later the regional lymph-nodes become infiltrated, and death occurs with the signs of general marasmus. Practically all such cancers arising on pre-existing arsenical keratoses are squamous-celled in type. The first microscopical changes in the malignant alteration of the keratoses consist of keratinization and vacuolization of individual cells in the epidermis (malignant dyskeratosis); further, the continuity of the basal layer becomes destroyed (see Montgomery, 1935, fig. 2*b* and 3). Arsenic can be found regularly by the Osborne method, and sometimes also by the chemical method (see p. 211, Chemistry and Histochemistry).

This type of arsenical cancer, the "Hutchinson type," is the earliest known, the most characteristic, and is very frequent. Nearly all cases reported until 1910 belong to it. Wile wrote still in 1912: "It is highly probable that all arsenical epitheliomata develop from keratotic nodules." About the frequency of this type see below, Frequency of Different Types.

2. *Arsenical epithelioma arising in a patch of psoriasis.*

In a few of the cases in which malignancy developed in cases of psoriasis treated with arsenic, the cancer originated from a psoriatic patch: cases 23, 32, 39, 43, 138 and possibly also 7 and 46.

The occurrence of cancer in cases of psoriasis attracted attention even before Hutchinson had discovered the carcinogenic property of arsenic (see above, p. 193). Hartzell (1899) collected a series of cases, and recognized that in nearly all cases there was evidence of prolonged arsenical treatment and presence of arsenical keratoses. So he arrived at the opinion that in these cases the cancer is a result of arsenical treatment and not of psoriasis. And as the malignancy nearly always developed on the keratoses, it was thought that all incidences of cancer in psoriasis were due to arsenic; not to the psoriasis. On the other hand Wile (1912) stressed the especially high incidence of psoriatics amongst the patients with arsenical cancer: "Considering the vast numbers of conditions . . . in which arsenic is employed, it seems more than a coincidence that of 19 cases, 15 have occurred in conditions (14 of them psoriasis and 1 pemphigus), in which an abnormality of epithelial growth ante-dated the use of the drug." In the same year Gray (1912) published a case of rodent ulcer originating in a patch of psoriasis (case 24). The patient had taken arsenic but without developing arsenical keratoses. Alexander, collecting all 17 cases of cancer in psoriatics, published until 1921, and adding one case of his own, tried to classify them in two groups:

> 1. Cases of true or genuine psoriasis-cancer ("echte Faelle von Psoriasis-krebs") in which a direct transition of a (mostly single) psoriatic patch in cancer is evident or very probable; here arsenic may not play an etiological role. There may be no history of ingestion of arsenic; keratoses may be absent. These cases are very rare.
>
> 2. Cases of arsenical cancer in psoriatics: The malignancy originates on an arsenical keratosis or on other (non-psoriatic) sites, often multiple

* Pictures, see Hutchinson, 1895 and 1903; Montgomery, 1935, etc.

LIBRARY
BOSTON UNIVERSITY
SCHOOL OF MEDICINE

from the beginning. History of arsenic ingestion; arsenical keratoses are present.

Remenovsky (1921), publishing in the same year a case of true psoriasis cancer (arsenic had been taken, but it seems after the beginning of malignancy), emphasized the extreme rarity of such observations.

Buergener, in 1939, in a critical review goes even so far as to say that a genuine psoriasis-cancer does not seem to exist. In nearly all the cases in which it may be supposed the influence of previous treatment with carcinogenic agents (arsenic, X-rays, radium) may have been of importance. In the remaining very few incidences there may be a chance of an accidental coincidence (two examples are given).

So even in these cases in which the malignancy has developed in a psoriatic lesion, arsenic as a causative agent may be assumed if arsenic has been given (cases 7, 24, 32, 39, 43, 46 and 138), or if its presence is proved by chemical or histochemical methods or if typical arsenical keratoses are found (cases 39, 43 and 46). On the other hand the coincidence of psoriasis and arsenical cancer —including the multiple benign superficial epitheliomas (see below)—is so frequent that some connection, e.g. a predisposition of psoriatics for cancer of the skin, is supposed by many observers (Wile, 1912).

Besides, it must be kept in mind that sometimes other lesions, especially erythematous plaques of superficial epitheliomatosis, are mistaken for psoriatic plaques (Pfahler, 1927; Montgomery, 1929; Wright and Friedman, 1933; Ellis, discussion to Anderson and Burpeau, 1945); even some forms of true cancer bear a striking clinical resemblance to lesions of psoriasis—psoriasiform basal cell carcinoma (see Sutton, 1939).

3. *Arsenical epitheliomas in normal skin.*

Wile wrote in 1912: "It seems highly probable that all arsenical epitheliomata had their starting-point in keratotic nodules; in no case is there evidence of ar epithelioma arising from the unbroken normal skin." This view cannot b. maintained further.

So in Lane's (1894) case (case 7) the first growth appeared on the forearm in apparently healthy skin. It seems that not seldom in the same patient some malignancies arise on keratoses and others on normal skin; but unfortunately precise notes on this point are missing in the majority of cases; frequently nothing is said about the previous appearance of the skin. Further, there is no doubt that the lesions of typical multiple superficial epitheliomas regularly develop in previously normal skin of the trunk and the proximal parts of the extremities (see below).

4. *Multiple (benign) superficial epitheliomata.*

(Multiple carcinoids, Arning, 1922; erythematoid benign epithelioma, Graham Little, 1923; epithelioma erythematoides benignum, Montgomery, 1929 and 1935; carcinoma cutis multiforme; epitheliomatosis.*)

This form of epithelioma of the skin and its relation to arsenic have attracted special interest since about 1930.

* Janeway as far back as 1910 gave a clinical and histological description of this type of epithelioma (Gross, 1931).

It is characterized by the eruption of multiple (usually numerous) superficial epitheliomas in the form of slightly depressed red patches mostly on the trunk and the proximal parts of the extremities, but neck and head, including the scalp, may also be affected. The lesions vary from ill-defined seborrhoea-like crusts to lesions several centimetres or even more in diameter with thick, verrucous, hard, dirty crusts. The removal of the covering crusts reveals a wide rolled indurated pearly border, in typical cases a very faint white wax-like narrow raised margin, flatter than the rolled edge of the lesions in the more malignant forms of epithelioma. (For pictures, see Montgomery, 1929 and 1941.)

Histologically the condition usually shows the picture of basal-celled epithelioma, but sometimes squamous-cell epithelioma and mixed types may be found (see below).

The affection is benign in character ; absence of ulceration over long periods ; long duration—10, 20, 30 years and even more ; the lesions sometimes disappearing spontaneously ; no metastases. Hence this kind of epithelioma apparently cannot be called a real cancer. But occasionally in the centre of a plaque a fungating squamous-cell epithelioma may develop, resulting eventually in metastases and death ; and besides, not infrequently they associate with other malignant kinds of epidermal growth, e.g. the Hutchinson type of arsenical cancer.

Graham Little (1923) has mentioned the fact that this condition also frequently affects psoriatics, and many others have confirmed this observation : Ormsby and Mitchell (1925), Stillians (1931), Senear (discussion to Stillians, 1931), Wright and Friedman (1933), Parkhurst (discussion to Arday, 1938), Applestein (1941), and Ellis (discussion to Anderson and Burpeau, 1945).

There is considerable controversy regarding the etiological role that arsenic plays in this type of epithelioma.

In 1931 Stillians and Gross presented patients with typical multiple epitheliomas who were treated previously with arsenic (cases 66 and 68). Andrews (1932), reporting another case (73), stressed this fact ; he admits that this fact alone is no proof for the etiological role of arsenic. On the other hand, he succeeded in his case in demonstrating arsenic in the tissue.

Anderson in 1932 reported three cases with exposure to arsenic (in the second case an occupational one), with keratoses of palms and soles ; in two of his cases (74 and 75) arsenic was detected in the urine ; in one (74) a small quantity also in the tissue. Anderson summed up all points speaking in favour of an etiological role of arsenic in the great majority of cases of superficial epitheliomas :

1. The frequent history of previous exposure to arsenic ; of course that is true for all cases of Table I, as this contains only arsenic-treated cases.

2. The frequent simultaneous occurrence of hyperkeratosis of the palms and feet, similar to that in the Hutchinson type of cancer. Among the about 46 uncombined cases in Table I, in 29 the presence of keratoses is stated (but only in 23 location of the keratoses on the hands and feet) ; in 9 cases their absence ; in 8 cases nothing is noted about keratoses. So the typical keratoses of hands or feet were present in at least 50 per cent of the cases. In one case (64) they started before the appearance of the epitheliomas, in case 74 later.

3. The fact that a considerable number of these patients had psoriasis and were treated for it, or for a condition mistakenly diagnosed as psoriasis (see above, pp. 200–201) Among the 46 patients with uncombined superficial epitheliomas in Table I, at least 21 were treated with arsenic for psoriasis. The question whether this treatment is responsible for the epitheliomas or if there is something in psoriasis itself that tends towards the development of these lesions was discussed in 1931: Senear cited two cases in which the ingestion of arsenic could be ruled out.

Pigmentation of the skin of arsenical type is noted in 9 out of the 46 uncombined cases in Table I.

In subsequent papers Anderson (1937, 1943*a*, 1943*b* and 1945) stuck to his opinion. In the discussion to Montgomery's lecture in 1941 he reported some 40 cases of superficial epitheliomas with arsenical keratoses of palms and soles; in one case at least arsenic was found in the urine. A similar case of Anderson and Burpeau (1945) is not included in Table I because X-rays were also administered.

Ayres (1934 and 1943), confirmed the views of Anderson by reporting two cases apparently caused by arsenic (85 and 131). Ayres and Anderson (1934) reported that in examining 44 patients with basal-cell epithelioma—most of them may be supposed to belong to this type—they found punctate keratoses in 75 per cent. Among 19 cases 12 were demonstrated to have arsenic in the urine, and in six others there was a history of exposure to arsenic.

Wright and Friedman (1933), reporting five cases of superficial epitheliomatosis in psoriatics, three of which had used arsenic, denied the etiological role of that drug because none of these patients had keratosis or pigmentation, and arsenic could not be demonstrated by Osborne's method.

Halloran (discussion to Anderson, 1937) and Traub (1937) supported the view of Anderson. Arday (1938) seems to doubt whether arsenic is a causative factor in such cases, whereas Parkhurst (discussion to Arday, 1938) assumes a relation. Cannon's (discussion to Montgomery, 1935) patients with multiple basal-cell epitheliomas gave in the great majority of cases a history of ingestion of arsenic, and had large quantities of arsenic in blood, urine and skin.

Montgomery (1935 and 1941), with the enormous experience of the Mayo Clinic, denied the etiological role of arsenic in true benign epitheliomatosis. He emphasized that the cases of multiple superficial epitheliomas have to be differentiated in two distinct groups:

1. The multiple benign superficial epitheliomatosis proper, characterized especially by a fine threadlike slightly elevated border of the lesions and by benign character, most of the lesions being of the basal-cell type (picture, Montgomery and Waisman, 1941, fig. 5). Amongst 65 cases of this group (16 described in 1929 and 49 in 1935) only one patient remembered to have taken arsenic. So Montgomery does not believe in arsenic as a definite cause of this condition.

2. The secondary form of multiple superficial epitheliomas arising also from non-keratotic areas and occurring also with predilection for the trunk, but marked by simulating lesions of arsenical keratosis, especially by a wide rolled indurated (not a threadlike) border, and tendency to malignancy (picture, Montgomery, 1935, pp. 223, 373, and 1941, fig. 4).

Histopathologically they are mostly squamous epitheliomas, but sometimes also basal-celled ones are found (Montgomery and Waisman, 1941). Nearly all cases of multiple superficial epitheliomas with a previous history of arsenic or with keratoses of palms and soles are put by Montgomery together with this group, setting them apart from true benign epitheliomatosis. Evidence of pigmentation he noted only in a few of these cases. Attributing to arsenic a very important etiological role he described these cases as " arsenical type of epithelioma," a name which may cause confusion with the keratoseogen Hutchinson-type of arsenical epithelioma. Montgomery reported 7 cases in 1935 (e.g. cases 103, 104 and 105) and 4 in 1941 (e.g. cases 127 and 128). Fraser (discussion to Wise, 1929) supported Montgomery's distinction of two groups of epitheliomas of the trunk.

Montgomery tried to prove his view by chemical analysis. In the growths of the second group he found regularly a considerable amount of arsenic by the chemical as well as by the Osborne method (cases 104, 105, 127 and 128). He lays stress especially on the fact that the growths contained more arsenic than the adjacent normal skin of these patients ; whereas in the lesions of the true benign group, in the great majority of cases none or little was found, in any case less than in the normal skin.

Montgomery does not deny any relation of benign epitheliomatosis to arsenic. He admitted that in some cases arsenic may be detected by Osborne's method (1935 and 1941). Further, he himself stated in 1935 that the two types of epithelioma may be associated in the same patient, as in 7 of the 16 cases of " arsenical type of epithelioma " (e.g. case 106). But he suggests that in such cases arsenic is not the cause of the benign epithelioma, but presumably only precipitates its development caused by an unknown predisposition.

There is no doubt that many of Anderson's and Ayres' patients belong to Montgomery's second group, the " arsenical type of superficial epithelioma " ; but at least some of their cases seem to be true benign epitheliomas. Anderson (1937) doubts whether the distinction between the two groups is really practicable ; he " cannot see how anyone can make such a hair-line distinction as regards the border."

Most authors agree now that at least a proportion of superficial basal-celled epitheliomas are caused by arsenic. Opinions differ mainly as to how great this proportion is ; whether nearly all incidences of this condition are due to arsenic (Anderson and Ayres), or many of them (Barber, 1939 ; MacCormac, discussion to Barber, 1939), or a certain percentage (Bloom, 1936 ; Gray, discussion to Barber, 1939) or only a few (Mumford, *ibid.*, 1939). Andrews (1932) thinks that arsenic may be supposed to play an etiological role, but there is no real proof. Barker (discussion to R. M. Montgomery, 1942) reported lately a high content of arsenic in the blood of such patients.

In a very high proportion of cases multiple superficial epitheliomas were associated with other forms of arsenical cancer (see p .207, Frequency of the Different Types).

Another type of epithelioma is mentioned by Cannon (discussion to Montgomery, 1935) : basal-cell epitheliomas on the face of comparatively young persons recurring at intervals of several years in different locations, with presence

of large quantities of arsenic. But no details of the histories are given, and the author himself finds it difficult to evaluate the presence of arsenic.

5. *Bowen's disease.*

Much confusion has arisen in the last twenty years about " Bowen's Disease " and its relations to arsenic.

Bowen, in 1912, described an extremely chronic affection of the skin with atypical epithelial proliferation as a new type of " pre-cancerous dermatosis." This Bowen's disease is marked clinically by multiple slightly elevated papules of dull-red colour, covered with scaly crusts, and arranged in groups—often with arciform configuration—or as multiple lenticular plaques. The histological picture is described as a neoplastic process with abnormal keratinization, " malign dyskeratosis " (Darier, 1914–15 and 1920), and amitotic cell division producing giant cells. The changes are often similar to those in arsenical keratosis, but vacuolization of the prickle cells is usually not so pronounced. The neoplastic process may remain intradermal, but sometimes an infiltrating squamous-cell epithelioma with metastases develops (in about 20 per cent of the cases). Fraser (1928)* pointed out that the cellular changes are characteristic of malignancy from the beginning ; therefore the condition has to be classified, not as a " precancerous " dermatosis, but as a real carcinoma, but without infiltrating properties : an intradermal cancer of the squamous-cell type. Wise (discussion to Fraser, 1928), Highman (*ibid.*,) and Ebert and Otsuka (1943) agree with Fraser.

The question whether Bowen's disease is a strict entity was always a controversial subject, especially whether a sharp line can be drawn against multiple superficial epitheliomas. Bosellini (1928) denied this. Anderson held the view that both conditions bear relationship to each other, their simultaneous occurrence in some cases testifying this view. Wise (1920), Eliascheff (*cit.* Barber, 1928), Montgomery (1929, 1935 and 1941), and Allington (discussion to Alderson, 1935) maintain that Bowen's disease is an entity and can be differentiated from multiple superficial epitheliomas. But Montgomery admits that both conditions are found occasionally associated, and that either can be associated with the ordinary arsenical type of epithelioma.

Wise (1928) and Fraser think that a definite diagnosis of Bowen's disease cannot be made on clinical evidence but only by histological examination ; Montgomery and Waisman (1941), on the other hand, state that the same histological structure is found also in other precancerous dermatoses. Montgomery (discussion to Goeckerman and Wilhelm, 1940) emphasized especially the fact that malignant dyskeratosis simulating Bowen's disease is a characteristic feature of the arsenical type of epithelioma. It is a matter of fact that almost no case presented during the last years in a dermatological society has escaped doubts about correct diagnosis (see Schoff, discussion to Alderson, 1935 ; Montgomery about case 74 ; Ebert and Otsuka (1943, discussion) ; and the frequent use of ill-defined terms such as " Bowenoid " or " Bowen-like " indicates the lack of evidence in that matter.

Anderson (1932), having observed a case of Bowen's disease with a history of arsenic, arsenic present in the urine, and keratoses of palms and soles (case 74),

* See also Lane, discussion to Ormsby and Mitchell, 1925.

expressed the opinion that this condition as well as multiple superficial epitheliomas are the result of this poison. Fraser (1927 and 1928) reported a similar incidence.

But H. Montgomery (1935) did not agree; in his five cases of Bowen's disease neither had arsenic been administered nor were keratoses or pigmentation present. He explains Anderson's case as an epithelioma of the arsenical type simulating the clinical picture of Bowen's disease.

R. M. Montgomery (1942) reported a case of Bowen's disease verified by autopsy in which arsenic was taken, with keratoses of fingers and toes (case 129).

Among the cases in Table I in only five the strict diagnosis of Bowen's disease was made by one or another observer (cases 55, 74, 103, 129 and 133); in three of them keratoses were present; in two association with multiple superficial epitheliomas; in one of these also with keratoseogen cancer and two cancers of mucous membranes. Besides, in seven incidences (67, 85, 109, 110, 121, 125 and 134) a " Bowen-like " or " Bowenoid " appearance is noted.

6. *Extramammillary Paget's disease.*

This affection, mostly localized on the genitals, seems to be very similar to, or identical with Bowen's disease (Fraser, 1928; Highman, discussion to Fraser, 1928; and Montgomery, 1929). The relations to arsenic are not clear, but may be the same as in Bowen's disease.

7. *Arsenical cancer in other organs than the skin.*

In some instances arsenical cancer of the skin is associated with cancer in other organs.

Thus one of Hutchinson's (1888) original cases died of cancer of the stomach (case 5). In one of Ullmann's cases (case 33), of Hutchinson type, a cancer of the tongue and later a cancer of the oral mucosa developed. It may be the same case which was mentioned by Mayer (1933). Franseen and Taylor (1934) refer to a case of keratoseogen cancer of the skin with a suspicion of associated cancer oesophagi (case 88).

Associated with multiple superficial epitheliomas and with typical keratoses a cancer uteri occurred in the case of MacCormac (1933*a*, *b*, case 76); with multiple epitheliomas and a squamous-cell epithelioma of the groin and a carcinoma of the tongue in case 123 (Anderson, discussion to Goeckerman and Wilhelm, 1940).

In a case of Montgomery (1935), with association of keratotic epitheliomas and numerous superficial epitheliomas (case 103) an intra-urethral epithelioma developed and later a bronchial carcinoma of " arsenical type."

Goeckerman and Wilhelm (1940) observed a papilloma of ureter and bladder in a case with keratosis of palms and soles and Bowen-like keratotic lesions on the trunk (case 122). In the discussion to Montgomery and Waisman (1941), Anderson mentions one case of Fraser and Fox and another of Goldman and Tauber, both with papilloma of the bladder. Barney (1944) reported a cancer of the oesophagus in a patient with keratoses and multiple superficial epitheliomas beginning in a patch of psoriasis (case 138).

It may be assumed that these localizations on the mucous membranes were also due to arsenic; several authors have made this suggestion (Ullmann, 1922; Goeckerman and Wilhelm, 1940; Montgomery, *ibid.*, discussion; Anderson, *ibid.*),

but it is not exactly proved ; a mere coincidence cannot be excluded. Arsenic never was identified in these growths until now.

In some cases after administration of arsenic, cancers in different organs occurred without cutaneous cancer but with arsenical keratoses ; thus a case of cancer of the breast published by Dubreuilh (case 22). Franseen and Taylor observed a cancer of the pancreas in a patient with typical keratoses (case 93).

In other cases after use of arsenic extracutaneous cancers occurred without keratoses or other signs of chronic arsenicism ; so in the cases of Milian (cancer of the breast, case 70), and Semon (1945) (case 142, cancer of the bronchi). The possibility of a mere coincidence of ingestion of arsenic and development of cancer is here of course much greater.

The case of Rosen (discussion to Montgomery, 1935, case 108) concerning epithelioma of the mouth after anti-syphilitic treatment with arsphenamines is already mentioned.

8. *Combination of types.*

Frequently all epitheliomatous lesions in a patient belong to the same type of arsenical cancer, e.g. several growths of the Hutchinson type. But in many other cases two or more different types are associated, as keratoseogen cancer with multiple superficial epitheliomas or with Bowen's disease. In some cases the keratotic cancer develops earlier (as in cases 103 and 128) ; sometimes the superficial epitheliomatosis (case 113). Lesions of the same type in a patient may develop in a different manner, e.g. one superficial lesion as a basal-cell epithelioma and the other as a squamous one. Further, there may arise additional cancers in other organs than the skin. So not seldom a very polymorphous picture is produced not seen in other kinds of growth ; as in the case of Guggenheim (1933, case 78), one cancroid, one basal-cell carcinoma and multiple atypical lesions with Bowen-like changes were combined with verruca seborrhoeica ; another example is the case of Montgomery (1935, case 103, Table I).

9. *Frequency of the different types of medicinal arsenical cancer.*

It is impossible to group every one of all the 143 cases of Table I in the different types mentioned above as the data are not always sufficient ; and besides, there are a number of cases presented in dermatological societies in which the experts did not agree in the special diagnosis. But in about 136 incidences a classification seems possible.

The total number of cases of the keratoseogen type may be assumed as 69.

Fifty-one cases belong apparently to the simple uncombined " Hutchinson type " ; in 36 of them the origin of the malignancy on a keratosis is definitely noted (cases 1, 2, 3, 4, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, 20, 21, 25, 26, 27, 29, 34, 36, 37, 42, 47, 57, 59, 60, 84, 87, 90, 92, 102, 112, 130 and 143) ; in the remaining 15 cases it can be assumed (cases 9, 19, 35, 40, 41, 46, 51, 56, 58, 62, 64, 71, 91, 94 and 126).

In 3 cases the Hutchinson type was associated with extra-cutaneous cancer (5, 34 and 88).

In 13 cases there was a combination with multiple superficial epitheliomas or with Bowen's disease (cases 11, 23, 49, 85, 104, 106, 107, 109, 111, 113, 116, 127 and 128) ; in 2 cases with multiple superficial epitheliomas and with extra-cutaneous cancer (103 and 123). 67 cases may be counted as multiple superficial

epitheliomas including Bowen's disease: 50 of them were uncombined cases (28, 30, 44, 45, 50, 52, 55, 64, 65, 66, 67, 68, 72, 73, 74, 75, 77, 78, 79, 80, 81, 83, 86, 89, 95, 96, 97, 98, 99, 100, 101, 110, 115, 117, 118, 120, 121, 124, 125, 129, 131, 132, 133, 134, 135, 136, 137, 139, 140 and 141); in 13 cases they were associated with the Hutchinson type (see above); in 1 case with extracutaneous cancer (case 75); in 2 cases with the Hutchinson type and with extracutaneous cancer (see above); in another case one of the multiple lesions had arisen in a patch of psoriasis and was associated with cancer of the oesophagus (see below). At the utmost five of these 67 incidences can be classified as cases of Bowen's disease.

Origin of growth in a patch of psoriasis is noted in 7 cases (7, 23, 32, 39, 43, 46 and 138); in case 138 it was multiple superficial epitheliomatosis originating in a patch of psoriasis; later a cancer of the oesophagus developed.

Special cases of growths of the skin are: one incidence of fibrosarcoma (case 53) and one of a melanosarcoma (?) followed by an epithelioma of the cheek (case 69).

The number of cases of extracutaneous arsenical cancer is 13. In 6 of these the skin was not affected (cases 22, 70, 93, 108, 122 and 142); in 7 there were also lesions of the skin (see above).

It is hardly worth while to calculate the percentages of the various types, as they depend also upon the interest the different forms have found at different times. Thus before 1931, of 39 incidences of the then modern keratoseogen type, only 7 of the then nearly overlooked multiple superficial type, Bowen's disease and their combinations with the keratoseogen type were reported. Since 1931 the interest switched over to the latter forms and the ratio of the published cases changed to 15 : 57. Of all cases of epithelioma arising from a psoriatic lesion all but one were reported before 1925.

10. *Multiplicity of medicinal arsenical cancer.*

Hutchinson himself emphasized that in arsenical cancer frequently several (primary) growths are present. Pye-Smith (1913*b*) in his collection of 29 cases found multiplicity of the lesions in fully half of all incidences. In Kennaway's table (1925) there are 75 tumours in 38 patients, giving an average of nearly two tumours in each patient, and besides, two patients had numerous lesions.

Now that many cases of the multiple superficial type have been published, the multiplicity appears to be even more pronounced. The calculation of the cases in Table I shows:

	Plain keratoseogen cases.	Multiple superf. and Bowen cases, plain and combined with other types.	All cases.
One lesion	24 = 44·4%	3 = 4·5%	41 = 28·7%
Two lesions	10 = 18·5%	3 = 4·5%	17 = 11·9%
Three lesions	6 = 11·1%	6 = 9·0%	12 = 8·4%
Several (4–6) lesions	13 = 24·1%	23 = 34·3%	38 = 26·6%
Numerous (10 or more) lesions	1 = 1·9%	32 = 47·8%	35 = 24·6%
Sum	54 = 100·0%	67 = 100·0%	143 = 100·2%

It must be kept in mind that multiplicity of lesions is quite frequent also in non-arsenical cutaneous epithelioma. In a review of 3000 cases of malignant tumours Owen (1921) found 143 (4·7 per cent) of multiple primary growths and not less than 113 of them involved the skin only. And Schreiner and Wehr (1934) report that the most common primary multiple tumour is the basal cell epithelioma of the skin, the squamous-cell epithelioma of the skin being the next in frequency. Hueper (1942) estimates that multiplicity in non-arsenical cutaneous cancer is only 5 per cent.

It is evident that in the simple keratoseogen cases the average number of growths is slightly over two; whereas in the multiple superficial cases with their various combinations more than one-third shows more than one lesion and more than one-half "numerous" or "several" lesions.

11. *Distribution of medicinal arsenical cancer.*

Hutchinson (1887 and 1888) laid stress on the frequency of location of the malignancies on the hands and feet. Wile (1912) stated a predilection for sites of frequent physiological or other trauma, in contrast to other cutaneous epitheliomas in which the face is the most common seat of disease. Following McCoy (1920) 37·7 per cent of all cancers of the skin occur on the face, hands and neck, all exposed to solar light. Kennaway in 1925 gave a review of the distribution in 38 cases published until this time, mostly of medicinal origin, including the cases collected in 1913 by Pye-Smith. He confirmed the "well-known tendency of arsenic to affect the fingers and toes" in contrast to cancers in tar, pitch and shale oil workers, showing an outspoken predilection for scrotum, head and neck. Among 75 arsenical growths in 38 patients:

46 per cent were located on fingers, leg, foot, toes and trunk.
23 per cent on arm and hand.
23 per cent on scrotum, penis, head, face, neck, eye and eyelid.
8 per cent on other parts.

Kennaway criticized the assertion of Bayet and Slosse (1919*a*, *b*) that the scrotum and its neighbourhood are sites of predilection for arsenical cancer; this opinion was based on statistics, omitting the so frequent localization on the hands.

Haagensen (1931), comparing the distribution of therapeutic arsenical cancer with occupational cancer, reported 4 new cases of the former with multiple lesions of the hands, arms, legs and trunk, but sparing the face and the scrotum (cases 62, 63, 64 and 65).

Since then many cases of multiple superficial epitheliomas have been added—partly pure cases, partly combined with other forms; these cases differ in their distribution essentially from those in the Hutchinson keratoseogen type. Therefore in Table II the cases of Table I are divided into several groups.

Table II confirms the preference of the keratoseogen Hutchinson type for the distal parts of the extremities, as nearly 85 per cent of the cases show one or more localizations on fingers, hands, toes and feet, and relatively few on other regions. In contrast more than 92 per cent of the multiple superficial cases presented lesions on the trunk (the cases with distribution "all over the body" included), and frequently also on the proximal parts of the extremities and in

more than one-third of the incidences on head and neck. The combined cases show a combination of both sites of predilection. Localization in the genito-anal region is relatively uncommon in both types. In the rare cases of epitheliomas originating from patches of psoriasis nearly always the more proximal parts of the extremities and the genito-anal region were affected.

12. *Chronological course of medicinal arsenical cancer.**

The time elapsing from starting the arsenical drug until the beginning of epitheliomatous growth lasts regularly many years but varies within wide limits: 3 years in case 130 and 40 years in case 74, average being 18·1 years. Even after the cessation of ingestion of arsenic there may be an interval of many years, as 18 years in case 15. There is no marked difference in the duration of this "latent period" between the different forms: in the Hutchinson type the average duration is 18·8 years, in multiple superficial and Bowen's type 17·8 years, in the combined forms 19·7 years.

In the cases with keratoses, especially in the Hutchinson type and in the combined type cases, this latent period may be divided into two stages, the first being the interval between starting with arsenical drugs and the first appearance of the characteristic keratoses; this prekeratotic stage, noted in 19 cases, lasted from one year† (in case 138) to 20 years (in case 104), with an average of 8·8 years (see above). The second stage is the time between appearance of the keratosis and the onset of epitheliomatous growth; this keratotic or precancerous stage, noted in only 11 cases, varies from one year (cases 4 and 29) up to 23 years (case 35) with an average of 9·6 years.

13. *Age at onset of medicinal arsenical cancer.*

In spite of the usual long duration of the prodromal period the age when the epitheliomas appear is relatively low—lower than in the common non-arsenical cutaneous cancer. This starts only seldom before the fortieth year of life, the average age being about 60 years (according to Pack and LeFevre (1930), 58 years for the squamous type and 61 years for the basal cell type). The earlier development of arsenical cancer already recognized by Hutchinson (1887 and 1888) was confirmed by all authors. Pye-Smith (1913*a*, *b*) stated that in one-quarter of the cases the patients did not exceed 35 years.

In calculating the age at onset of neoplastic growth in the cases of Table I again the difficulty arises that in a great number of cases accurate dates are missing. Among 115 cases (80 males and 35 females) the age of which is noted, the start of neoplastic growth can be fixed approximately (see Table III). About one-third of all cases were not older than 40 years, and more than 60 per cent not older than 50 years. Remarkable is the considerable number of females between 20 and 30 years.

In one case ("case 2" of Oliver and Finnerud (1928), not included in Table I on account of the absence of other details) the cancer appeared in a boy of 11 years.

* Duration of exposure to arsenic, see above.

† Appel (discussion to Lane, 1939) reports that in one of his cases arsenical keratoses developed after only 3 weeks' use of arsenic, and Montgomery (1935) has seen keratoses one month after starting the drug.

IX. Histology of Arsenical Cancer.

Both main types of skin cancer are observed in arsenical malignancy :

1. The squamous-cell epithelioma = prickle cell ep. = spinocellular ep. = pearl forming ep. = epidermoid carcinoma, differentiating into all the Malpighian layers ; it is marked by a tendency to cornification (" horny pearls " or " cell nests ") and a higher degree of malignancy (metastases in lymph-nodes) ; it is the usual form in arsenical epitheliomas arising on keratoses.
2. The basal cell epithelioma of Krompecher = basilioma = rodent ulcer type, starting from the basal layer of the epidermis, with no tendency to cornify and comparatively benign course (no tendency to metastases), although it consists of younger and less differentiated cells than the squamous type. It is the most frequent form in multiple superficial epitheliomas.

There are also mixed forms and transitions from the basal-celled to the squamous-celled type. Besides, both types of epithelioma may occur in the same patient.

For the histology of Bowen's disease and extramammillary Paget's disease see above.

Among 81 cases in Table I with cutaneous tumours in which the result of histological examination is noted, at least 44 were of the squamous type and at least 24 were basal-celled ; one was a mixed form. In 10 cases squamous-cell tumours as well as basal-cell tumours were present. Only in one case (129) the growths were microscopically exactly identified as Bowen's disease. In one case (case 53) a fibrosarcoma and in one case (case 69) a melanotic sarcoma is noted.

X. Chemistry and Histochemistry in Arsenical Cancer.

Arsenic after ingestion for a long time is said to be deposited in the organs, especially in the skin and its appendages (sweat glands, sebaceous glands, hair follicles, hair and nails). Partly it is excreted—very slowly—by these glands (see Cornbleeth, discussion to Montgomery and Waisman, 1941) and in the urine. For chemical investigation in the living are available : urine, blood, spinal fluid, hair, nails and excised samples of the normal and pathological skin.

The methods of chemical examination are based on the classic method of Marsh. Now mostly used is the electrolytic technique of Gutzeit (1879) or of Osterberg (1928). Table IV gives the results of the authors in cases of arsenical epitheliomas in the urine.

The value of such investigations of the urine for the diagnosis and for the clearing up of the pathogenesis is by no means as high as should be expected. It must be kept in mind that arsenic in small amounts is apparently a normal constituent of the body, depending on environmental conditions, as water, food and occupation (Heffter and Keeser, 1927 ; Rost, 1931).

Thomsen (1905 *cit.* Schwarz, 1932) was the first to detect arsenic in the normal urine. Billeter and Marfurt (1923) found 0·00056 mg. per cent and 0·0104 mg. in the daily quantity ; Althausen and Gunther (1929) 0·08 and 0·16 mg. per cent ; Throne and Myers (1935) 0·00104 mg. per cent ; they think an amount of over

0·03 mg. per cent of dried substance to be pathological. Wuehrer (1937) found 0·005–0·095 mg. per cent in normal urine. In the urine of (healthy) workers with arsenicals Schwarz (1932) detected amounts up to 0·82, and Wuehrer in cases of chronic arsenicism (without epitheliomas) 0·245–1·110 mg. per cent As_2O_3. So practically all quantities found in the urine of patients bearing arsenical epitheliomas (Table IV) are within the limits sometimes contained in the urine of apparently normal individuals.

It may be mentioned that after ingestion of arsenic the urine contains arsenic partly as an organic compound (Salkowski, 1908).

Some authors report determinations of arsenic in *blood* (Cannon, discussion to Montgomery 1935, and Montgomery, 1942) but no exact quantities are given in cases of arsenical cancer.

Still less successful than the examination of the urine proved the investigation of *hair* and *nails* in cases of arsenical growths. Caspar (1860) first described the presence of arsenic in the hair of a patient in whom arsenical poisoning was suspected. Since then many determinations in the hair were made (Schwarz, 1932; Tost, 1931; and Wuehrer, 1937). The values in the hair of normal persons lie between 0 and 0·18 mg. per cent (Myers and Cornwall, 1925; Billeter and Marfurt, 1923; Althausen and Gunther, 1929; Wuehrer, 1937), whereas after ingestion of arsenic and in (healthy) workers with arsenical insecticides amounts of 0·245, 1·110, 5·0 and even 160 mg. per cent (Schwarz and Deckert, 1943) were reported. It seems strange that investigation in three cases of arsenical cancer (Montgomery, 1935, cases 96, 97 and 98 of Table I) did not reveal any arsenic in the hair.

In the nails where normal amounts of 0·0172 mg. per cent were stated by Billeter and Marfurt (1923), Hamilton (1921, case 36) found only a trace.

In normal *skin* Billeter and Marfurt (1923) detected 0·0097 mg. per cent, and Oppenheim (1931) in the scales of normal skin 0·0026 mg. per cent. The examination of the keratoses, growths and normal skin in cases of arsenical cancer has given repeatedly negative results; as in the incidences 79, 101, 102 and 120 of Table I; in others amounts up to 8·6 mg. per cent (case 104) and even 430 mg. per cent (case 127) were recorded (Table V).

The results of chemical determination are disappointing on the whole. The main cause may be that—except for urine—usually only very small samples of tissue are available. It seems that the methods of determining arsenic in such small quantities are still unreliable. For this reason the histo-chemical method is preferred by the majority of authors. This "hydrogen sulphide method" has been worked out by Justus (1905), following a proposal of Carlson; the arsenic of the tissues is transformed to arsenic trisulphide As_2S_3, which can be recognized microscopically as yellow-brown to greenish-yellow crystals of high refractivity, nearly always of uniform size. The original technique has been modified by Bruenauer (1921) and Osborne (1925, 1928 and 1932). Osborne's technique is now mostly used. As a microscopical method of course it cannot give real quantitative values, but it is very sensitive, requires a minimal quantity of material and permits a location of the arsenical deposit. The method is supported by a series of authors: Montgomery (1935), Montgomery and Waisman (1941), and O'Leary (discussion to Waugh and Scull, 1935). But it has not escaped criticism. Muir and Tannenholz (1933) objected, stating that the crystals were in reality sulphides of albumin. Other authors are also critical concerning

the proof of arsenic by the histo-chemical method: Oppenheim and Fantl (1934), Becker (discussion to Waugh and Scull, 1935), and Nomland (*ibid.*). In any case the method requires fully experienced qualified investigators.

Bruenauer and Osborne obtained always negative results in the skin of normal persons and positive in arsenical keratoses.

Anderson (1932, case 69) and Goeckerman and Wilhelm (1940, case 114) reported positive results with Osborne's method. But the most extensive use of examining normal and pathological skin of the patients by chemical and histochemical methods was made by Montgomery (1935, 1941) (Table V). Montgomery himself emphasizes that arsenic in pathological amounts can be found by Osterberg's method in cases of various types of dermatoses without there being any clinical evidence of arsenic, as keratoses or pigmentation. On the other hand, neither he nor Osborne was able to demonstrate arsenic in the skin of certain patients who had got arsenic for some time, even in some cases with arsenical keratoses or typical arsenical cancer. So the mere demonstration of arsenic by chemical or histochemical methods does not prove that the drug is an etiological factor in a given case; and its absence does not exclude arsenic as a causative factor. O'Leary (discussion to Waugh and Scull, 1935) has confirmed these observations.

Cannon (discussion to Montgomery, 1935) may be right in saying that it is necessary to learn more about the significance of arsenic in normal persons.

Guthmann (1938) reported that he had found in the venous blood of normal women 0·01–0·06 mg. per cent, with considerable variations corresponding to the menstrual cycle, and still higher amounts up to 0·24 mg. per cent in pregnancy. In cancers of different origins he found quantities of 6–7 mg. per cent, together with an increase in the venous blood up to 4½ times the normal value. He suggests a close general connection of arsenic with different proliferation of cells. But his values are so astonishing and in such apparent contradiction to the results of other authors that confirmation would be necessary (see p. 229, Pathogenesis).

XI. Prognosis of Arsenical Cancer.

As with cutaneous cancers in general, arsenical epitheliomas of the skin are slowly growing neoplasms with a relatively low grade of malignancy, the microscopically basal-celled forms giving a better prognosis than the squamous ones. Besides, there are obvious differences between the different clinical types.

The keratoseogen cancers of the Hutchinson type—nearly always squamous forms—although often growing very tardily at first, give the most unfavourable ultimate prospect; especially the cases originating on the fingers. They lead eventually to metastases in the regional nodes and in the internal organs, to emaciation and death; the multiplicity of lesions is another aggravating factor. Such tumours localized on other sites seem to have a somewhat less unfavourable course.

Much better is the prognosis in superficial epitheliomas and in Bowen's disease; but there are also differences. The most favourable are the cases of true superficial "benign" epitheliomatosis showing the histological picture of basal-celled epitheliomas, and often without any sign of keratosis or pigmentation. They cannot be called real cancers, but occasionally they become malignant (e.g. case 139, discussion to Glen, 1945), or they are associated

with other malignant forms of epithelioma. Less favourable are the cases of superficial epitheliomas with marked keratoses (Montgomery's "epithelioma of the arsenical type"), frequently showing the histological picture of squamous-cell epithelioma; they are usually not so malignant as the keratoseogen forms, and Montgomery and Waisman (1941) suggest that the clinical course is frequently not so bad as the histological picture would suggest, but nevertheless they often lead to invasive growth, metastases and death.

In Bowen's disease (see p. 205) the lesions may be constant for many years, although the microscopical picture is like squamous cancer. Approximately 20 per cent eventually develop into a penetrating form of cancer.

Death is noted in 17 cases of Table I, but no doubt the number of fatal cases is in reality far greater.

XII. Treatment of Medicinal Arsenical Cancer.

There is general agreement that the main task in therapy is prophylaxis. That means, in medicinal arsenical cancer, to confine the use of this powerful drug to the cases of necessity. Arsenic seems to be dispensable as a tonic and as a preventive for bromide eruption in epilepsy, etc. (its efficiency for that is even doubtful, see Wigley, discussion to Russell and Klaber, 1945). That applies especially to the treatment of psoriasis, as patients suffering from this disease are apparently more susceptible to the different kinds of arsenical epithelioma. The question was specially discussed in connection with Anderson's paper (1937). Some authors (Ayres, discussion to Anderson, 1937; Netherton, discussion to Barney, 1944) suggest that the benefit obtained by arsenic in psoriasis does not justify the risk of administering it, or only in exceptional cases. Caskey (discussion to Anderson, 1937) declared he never mentions arsenic to his students or physicians without condemning it. Others (e.g. Jacobsen, discussion to Anderson, 1937) do not go so far; for arsenic is doubtless very effective in many cases of psoriasis—testified by the very histories of some patients of Table I (see cases 1, 23, 24, 38, 43, 120 and 130); and it must be considered that in comparison with the high frequency of psoriasis and the general use of arsenic for its treatment the development of arsenical cancer is quite a rare event. In the therapy of lichen planus and of dermatitis herpetiformis also arsenic cannot be spared easily. Halloran (discussion to Anderson, 1937) declared it would be a mistake to develop a fear complex as to the use of arsenic as a drug. But certainly there should be legislation to prevent or curtail the general use of arsenic in patent medicines, and it should not be permitted that a patient can get the drug continuously without a new prescription. Repeatedly it was claimed in the discussions that the prescription must have the remark, "Not to be refilled." It is equally necessary to instruct students about the danger of arsenical medication, and the indispensability of a regular control of patients treated with arsenic. The use of arsenical paste for the treatment of cancer should be abandoned, even although it has proved in some cases to be successful (see picture in Sutton, 1939, p. 729).

Ullmann and Galloway (discussion to Pye-Smith, 1913*a*) sounded a note of warning that the use of arsphenamine in large quantities might also be followed by malignant epithelial lesions. But experience has shown that there is not sufficient reason to restrict such a valuable drug in the treatment of syphilis, as

the development of malignancy after its use is very exceptional (see p. 196), and such patients are as a rule under medical supervision. Only in cases of syphilitic affection of the mouth, especially leucoplakia, a certain reserve may be recommended (Rosen, discussion to Montgomery, 1935).

As soon as signs of chronic arsenicism (pigmentation, keratosis, etc.) begin to appear, the medication with arsenic has to be stopped at once. The use of sodium thiosulphate was recommended: 5–10 c.c. of freshly prepared 10 per cent solution intravenously every third day or orally 0·5 g. in enterically coated tablets. Ravaut (1920) and Dennie and McBride (1924) have introduced this treatment. It is supposed that this salt converts arsenic in the body into the insoluble harmless sulphide (see Throne and Myers, 1935). Some authors (Ayres and Anderson, 1938) believe that it increases the excretion of the poison. Many authors support this treatment: Althausen and Gunther, 1929; Throne and Myers, 1935; Greenwood (discussion to Lane, 1939). But new investigations have shaken its theoretical basis (Editorial, 1942; Abramowitz, Mattice and Bottvinck, 1944). Appel (discussion to Lane, 1939) recommends the external local use of sodium thiosulphate in the form of fairly concentrated soaks. Also, soothing ointments (hydrous wool fat and petrolatum) or keratolytic ointments (with salicylic acid) may be applied for removing the keratoses. Franseen and Taylor (1934) recommend prophylactic destruction of the keratoses already in the pre-cancerous period. But it must be kept in mind that any irritation, mechanical or chemical, must be avoided (see p. 229, Pathogenesis). Of course regular medical supervision is necessary.

If a case of keratosis is developing signs of malignancy, radical measures have to be taken at once; they consist either of surgical removal (excision), or destruction by one or the other forms of cautery. In cancerous lesions of the fingers, and in extensive lesions of other parts of the extremities, amputation is advisable. It has been recommended in all cases of squamous cancer to remove the regional nodes too (Franseen and Taylor, 1934).

The more conservative methods of treatment such as radiotherapy (X-rays, radium) have proved insufficient, and even contra-indicated in keratoseogen arsenical lesions; recurrences were observed very often (Montgomery and Waisman, 1941). Some authors recommend radiotherapy as an after-treatment after surgical removal; but others (Galloway, discussion to Pye-Smith, 1913*a*) think that radiotherapy should be avoided entirely in the treatment of arsenical cancer.

For the treatment of multiple superficial epitheliomas and Bowen's disease it was thought that less radical procedures are justified, such as the use of X-rays, radium, ultraviolet irradiation (Ayres, 1934), or "Grenzstrahlen" (Kindler, discussion to Glen, 1945). But already Graham Little (1923) had noted the marked radio-resistance of these lesions, and other authors have confirmed this observation (Lane, discussion to Ormsby and Mitchell, 1925; Anderson, 1932; Glen, 1945; Dowling, discussion to Glen, 1945). Graham Little recommended freezing with carbon dioxide snow, already proposed by Galloway (discussion to Pye-Smith, 1913*a*); Schamberg (discussion to Pfahler, 1927) and Crawford (*ibid.*), electro-desiccation; Rodgers (1941) used cauterization with silver nitrate; Pusey (discussion to Ormsby and Mitchell, 1925), painting with nitrate of mercury (Weber, *ibid.*). But most of the experts now advise complete excision also for this kind of epithelioma, if necessary with skin grafting (Lane,

discussion to Ormsby and Mitchell, 1925 ; Michelson, discussion to Fraser, 1928 ; Montgomery, 1929 ; Glen, 1945 ; Dowling, discussion to Glen, 1945). Of course there may be difficulties in cases with numerous lesions. Ayres (1934) recommended after-treatment with sodium thiosulphate against further development of epitheliomas.

Of course, periodical re-examination of the skin is essential.

B. ARSENICAL CANCER CAUSED BY DRINKING-WATER.

As early as in 1809 Lambe (Eggers, 1932) expressed the belief that arsenic in potable water may be the cause of malignant disease.

One of the most impressive examples of an endemic occurrence of chronic arsenicism is the so-called *Disease of Reichenstein* (" Reichensteiner Krankheit "). Reichenstein is a small town in the county of Glatz, Silesia, where for many centuries gold was produced from gold-containing arsenical ores : arsenopyrite (= arsenkies mispickel FeAsS) and lollingite (= arsenikalkies = arseneisen $FeAs_2$ respectively Fe_2As_3 and Fe_2As_5). With the old methods of smelting large quantities of arsenical fumes escaped, were precipitated with the rain and thus reached the underground water, the brooks and the drinking-water. In one of the brooks (" Giftbach " = poisonous brook) 1·22 mg. per cent arsenic was found ; in samples of the mud up to 500 mg. per cent. Later the gold production was dropped and only arsenic was produced. For centuries the high incidence of serious diseases in that town was known. But not until 1898 was the nature of this disease recognized as chronic arsenic poisoning, and described by Geyer. The main symptoms were : troubles of the gastro-intestinal tract, ulcers in the mouth, perforation of the nasal septum, paraesthesia, and especially melanosis and keratotic warts on fingers and hands of the same appearance as in arsenical poisoning. In some cases cancer developed. Geyer refers to the reports of two local practitioners giving details of three cases of this kind, all with keratoses of the hands and melanosis. It seems that such cases were very frequent in Reichenstein ; for Kathe (1937) was told by another doctor there that half of his patients with " Reichenstein disease " had died with cancer. Unfortunately written records are missing.

The " Reichenstein disease " has disappeared since the methods of smelting have been changed, and particularly since the town was provided with a new and adequate water supply in 1928, with an arsenic content of at the most 0·0015 mg. per cent (Geyer, 1940).

Another occurrence of endemic arsenical intoxication caused by drinking-water is reported from the Argentine, province of Cordoba. In a wide area in the south and east of this province, including the town of Bellville, such cases have been observed by Goyenchea, by Ayerza and Arrillaga and others (Fernandez, 1925 ; Bosco, 1925 ; Roffo and Correa, 1926 ; Alvarez and Ruiz, 1927 ; Alvarez, 1928 ; Garcia and Ruiz, 1928 ; Alvarado, 1930 ; Seminario and Alvarado, 1931 ; Roffo and Rosner, 1935 ; Trelles, 1937 ; Arguello, Cenget and Tello, 1938 ; Arguello, Ferraris and Tello, 1938 ; Arguello, Tello and Marcola, 1942 ; Zinny and Vivaldo, 1942 ; Arguello and Tello, 1943). The drinking-wells of Bellville have been found to contain an abnormally high amount of arsenic, up to 0·28 and 0·45 mg. per cent ; iodine and vanadium are also present in many of these waters. Many cancers of the skin have been observed in Bellville,

mostly in peasants. Arguello and co-workers alone give reports of 65 cases. The clinical picture is in accordance with that of cancer caused by arsenical drugs: keratoses of the palms and soles seem to be present in nearly all cases, as in all 39 cases reported by Arguello, Cenget and Tello (1938); often melanosis—in 12 per cent of the cases in the mentioned paper. There is also a tendency to multiplicity and a predilection for the limbs, especially hands and feet, and a relative rarity of localization on the head. Among their 39 cases Arguello and his co-workers found multiplicity in 33·3 per cent; localization on the limbs in 38 per cent; on the trunk in 13 per cent; on the head in only 15 per cent; in no case on the genitals. The corresponding percentages in non-arsenical cutaneous cancer was found by the same authors to be: multiplicity in 5 per cent, site on the limbs in 4 per cent, on the trunk in 2·5 per cent, on the head in 81·5 per cent, and on the genitalia in 5 per cent.

The majority of the epitheliomas were of the squamous cell type (51 per cent); less frequently of the basal cell type (7·6 per cent). In a second paper Arguello, Ferraris and Tello (1938) report 26 new incidences, including three cases of Bowen's disease supporting Anderson's view that Bowen's disease is a form of arsenical intoxication (see p. 205).

There are several wells in Europe with a very high content of arsenic, frequently used for therapeutical purpose, as:

Guberquelle (Bosnie)	. . .	0·6	mg. per cent arsenic.
Levico (Tyrol)	. . . . .	0·6	,, ,, ,,
Duerkheim (Palatinate)	. . .	1·74	,, ,, ,,
Roncegno (Tyrol)	. . . .	4·26	,, ,, ,,

for comparison:

Bellville in the Argentine	. . .	0·28–0·45 mg. per cent arsenic.
"Giftbach," Reichenstein	. . .	1·22 mg. per cent arsenic.

In the Report of the Royal Commission on Arsenical Poisoning (1903) the limit of arsenic for liquid food (and water) was set up as one-hundredth of a grain of As_2O_3 per gallon = 0·015 mg. per cent. Nothing is known of a more frequent occurrence of cancer in these localities either at the present time or in the past.

Summarizing, it can be stated that cancer caused by the arsenic content of drinking water is apparently identical with medicinal arsenical cancer. In Cordoba not only the keratoseogen type but also the multiple superficial type and Bowen's disease were observed; that they are not mentioned in Reichenstein is not astonishing, as at that time these affections were not known. It seems that in Reichenstein melanosis was very frequent, and more pronounced than usual in the medicinal cases; besides, perforation of the nasal septum is mentioned as a frequent sign, not hitherto observed after ingestion of arsenic as a drug. But these are minor differences.

C. OCCUPATIONAL ARSENICAL CANCER.

I. Arsenical Cancer in Miners and Smelters.

The first hint that arsenic may be the cause of development of cancer in man is to be found in the *Pharmacologia* of Dr. J. A. Ayrton Paris. Having

practised in Penzance (Cornwall) from 1813–1817, he asserts that the smelting works developing arsenical fumes in Cornwall and Wales occasionally cause in the workers cancerous disease of the scrotum similar to that which affects chimney sweeps. This remark of Paris, quoted over and over again, was criticized by Butlin and by Kennaway.

Butlin (1892), making careful inquiries of the doctors in Camborne and Hayle, failed to find the smallest evidence confirming the assertions of Paris; the doctors practising there had never seen a case of scrotal cancer. A later inquiry by Hulke (discussion to Lane, 1894) amongst the workers in the Cornwall mines as to the occurrence of cancers also had a negative result. These differences could perhaps be explained by the fact that the method of smelting since Paris' time had changed, producing now mainly tin and developing less arsenic fumes. But that would not meet the objection pointed out by Kennaway (1943) that the arsenical cancer prefers other locations, affecting the scrotum very seldom. Out of 75 cases of arsenical cancer reported in the literature only 4 were localized there.

The question has been discussed as to whether the so-called "*Schneeberger Lungenkrebs*" and the pulmonary cancers observed amongst the miners of *Joachimsthal* are due to arsenic. For centuries the high mortality rate amongst the miners in Schneeberg and in other districts of the Saxonian Erzgebirge (ore mountains), as Annaberg, etc., has been known. It was mentioned by Agricola (1556) and in chronicles as due to "Bergkrankheit" (mountain disease). But not until 1879 was the condition described by Haerting and Hesse as cancer of the lungs. Arnstein (1913*a*, *b*) stated that they are real carcinomas, not lymphosarcomas or endotheliomas, as was suggested by Wagner and Weigert. Haerting and Hesse stated that 75 per cent of all deaths of the miners was due to this affection—later statistics show 40–70 per cent (Arnstein, 1913*a*, *b*; Lange, 1935). The onset of the disease in this district occurred as a rule after 20 years of working in the mines.

As other persons in this district were affected very rarely, this kind of cancer must be in some way connected with the mining. Haerting and Hesse assumed the cause of the disease to be irritation caused by inhaled arsenic with other respiratory affections and a bad state of nutrition as predisposing factors. The main ores in Schneeberg—besides sulphidic ones—are arsenides, as "Speiskobalt" (tin white cobalt $CoAs_2$), "Weissnickelkies" (white nickel $NiAs_2$) and "Rotnickelkies" (arsenical nickel NiAs); these compounds are more soluble than the compounds of arsenic and sulphur present in other cobalt pits. The boredust of the mines contains up to 0·45 per cent As (Rostoski, Saupe and Schmorl, 1926), the dust in the pits 0·1 per cent. For the importance of arsenic in the etiology of the Schneeberg cancer Saupe (1930) laid stress on the experimental production of pulmonary malignancy by Fischer-Wasels (1929, see p. 228) and Schmorl's (*cit.* Saupe, 1930) finding of two cases of lung cancer in the necropsies of workers in the forges of Freiberg-Muldenhuetten, where now arsenic is produced from different ores. But Saupe himself (1930) was unable to detect more instances of lung cancer in Freiberg, although sometimes signs of chronic arsenicism were found. It may be remembered that—why, is not known—in the last ten years cancer of the lungs, formerly rare, has become a quite frequent disease.

Considering all facts arsenic can hardly be said to have an important etiological

role in the Schneeberg cancer. No other signs of arsenicism are reported in these patients, especially not keratoses. The localization of cancer in the lungs is extremely rare in typical arsenical cancer: out of 143 medicinal arsenical epitheliomas of Table I the lungs and the bronchi were affected only in two cases (103 and 142). On the other hand, in the Schneeberg cases, although eczematous changes occurred frequently, never a cancer of the skin has been reported. Multiplicity of the tumours, so frequent in true arsenical cancer, is usually missing in the Schneeberg malignancies (Hueck, 1939/40). The tests for arsenic in urine, hair and nails gave negative or doubtful results (Rostoski, Saupe and Schmorl, 1926), and so did the examination of the lungs (Pirchan and Sickl, 1932, in Joachimsthal miners, and Ziel, 1935). Besides, pulmonary cancer is not very frequent in other parts of the world where arsenic is mined.

Other factors blamed for causing the Schneeberg cancer are: pneumokoniosis, chronic irritation by respiratory diseases, cobalt and hereditary susceptibility.

Now the general view is that the main etiological factor is the presence in the pits of radioactive substances, the carcinogenic action of which is well known (Ludewig and Lorenser, 1924). Also it was stated that in Joachimsthal, on the southern side of the Erzgebirge, famous for its content of pitchblende and radium, lung cancer is also found (Loewy, 1929; Sikl, 1930). In the necropsies of the miners there between 1928 and 1938 summarized by Peller (1939), in about 50 per cent lung cancer was found. In contrast to that, in the mines of Southern Sweden containing Speiskobalt, as in the Schneeberg mines, pulmonary cancer is unknown.

Recently Lorenz (1944) arrived at the opinion that radioactivity cannot be the sole cause of the pulmonary cancer of the miners, because similar doses of X- or γ-rays do not produce lung tumours in animals. He suggests some contributory factors, including arsenic.

Although there is no basis to suppose that arsenical cancer is widespread in miners and smelters, that does not deny that such cases occur occasionally. Some cases are reported in the literature: the case of Anderson (1932, case 1 of Table VI), concerning a man of 51 who as a boy had worked in a smelting works in Mexico, and developed warty keratoses of palms and soles and multiple benign superficial basal-celled epitheliomas; in the normal parts of the skin arsenic was detected, and still more in the lesions. The patient knew the lesions were due to arsenic. He stated that "everybody in the Mexican mines has the same thing."

Another case was reported by Migayi (1935): after this man had worked for ten years in a copper smeltery in Japan in which arsenic was produced, keratotic changes appeared on the soles and palms, pigmentation of the skin, and some years later a cancerous ulcer (squamous) developed on a keratosis.

A third case is that of Schaerrer (1934)—a worker mining or smelting silver ores.

A fourth case was mentioned by Andrews (discussion to Goeckerman and Wilhelm, 1940)—a miner in a copper mine, with typical keratoses and epitheliomas.

Also Bayet (1930) cites Rayer, who reported some cases of arsenical cancer among workmen employed in the handling of arsenical ores.

II. Arsenical Cancer in Factories.

1. *Sheep dip factories.*

Legge (1902) described, from a sheep dip factory in England, incidences of irritation of the upper air passages combined with keratosis of the skin and pigmentation as indicative of arsenic poisoning. Sheep dip is a compound of arsenite of soda and free arsenious acid used for the treatment of mange in sheep. The product is very dusty. Eve stated in 1910 that all workers at the factory were deeply pigmented (O'Donovan, 1928).

Pye-Smith (1913*b*) in his table of 31 cases of arsenical cancer recorded two until then unpublished incidences of cutaneous cancer in persons employed for years in such a factory, observed by Eve and by Porter. These observations were verified by later experience. Legge (1923, 1924) reported four similar cases. O'Donovan, describing again Porter's and one of Legge's cases, gave a review of the facts. Bridge (1926, 1930) mentioned three further incidences. Legge (1934), repeating apparently three of the former observations, reported another doubtful case. Merewether (1943) detected the incidence of fatal pulmonary cancer in a sheep dip worker, and remarked that 3 similar cases occurring in sheep dip workers had been notified since 1939. Currie (1947) found columnar-celled adenocarcinoma in the lung and bronchial lymph glands in case 15, Table VI. It is not noted whether these patients have shown any signs of chronic arsenicism, especially keratoses. So this matter needs further examination. This amounts to 13 cases of arsenical cancer in sheep dip workers all observed in England (Table VI). Arsenicism in sheep dip workers has become rare since the plants were remodelled to render them as nearly dust-free as possible.

2. *Cancer caused by arsenical insecticides.*

Chronic arsenicism has been observed in workers employed in the manufacture of arsenical insecticides. There are a great number of different preparations, with trade-marks such as Emeraldgreen, Silesiagreen, Titaniagreen, Urbangreen, Aresin, Esturmit, Vinuran, Grelit, Meritol, Nosprason, etc. Most of them contain as the main compound aceto-acetate—arsenite of copper = Schweinfurt-green $Cu(C_2H_3O_2) + 3Cu(AsO_2)_2$; others, arsenite of copper; Scheele's green $CuHAsO_3$; others calcium arsenate or lead arsenate. Schwartz and Deckert (1943) found very large amounts of arsenic in the hair of these workers, 74 to 160 mg. per cent (see p. 212).

The arsenicism in these workers may lead to the development of cancer; two incidences were published, one by Legge (1925) and by Bridge (1928), another by Bridge (1939); the latter with a pulmonary cancer besides skin manifestations (see Table VI).

These arsenical insecticides are especially used by gardeners, fruit farmers and vintagers for combating peronospora. They are used partly in the form of a powder and partly as a spray. It is no wonder that chronic arsenicism has been found also in such workers (Doerle and Ziegler, 1930).* Hyperkeratosis of the

* Aschoff (1887–1899) was the first who brought attention to the high frequency of cutaneous cancer in gardeners; this observation was confirmed by Young and Russell (1926), who stated that in gardeners, nurserymen, seedsmen and domestic gardeners the percentage of death from cancer of the face is 150 per cent in excess of the expected amount, deaths from cancer of the hands even 300 per cent. Exposure to sunlight, contamination with fertilizers (soot !) were held responsible; but handling of arsenical insecticides surely plays also a role.

skin with multiple warts and melanosis are the most conspicuous symptoms (Pein, 1938*a*, *b*). In some of such cases cancer of the skin developed (see Table VI, cases 20–23). All four cases showed keratoses, two melanosis. But none of them presented the true Hutchinson type of skin cancer. The cases 21, 22 and 23 may be grouped as multiple superficial epitheliomas. Case 20 shows an uncommon localization all over the face ; it may belong to the type mentioned by Cannon (discussion to Montgomery, 1935) (see p. 204).

Pein and Baurhenn (1943) believe that the high incidence of arsenicism in wine-dressers in Germany is not really occupational, but is due to the consumption of wine at home (" home-drink "). This may contain 0·1–0·2–0·5 mg. per cent arsenic, i.e. up to 2–12 mg. daily.

3. *Arsenical cancer caused by coal and its by-products.*

Cancer of the scrotum in chimney sweeps is well known since its description by Pott (1775). Volkmann (1875) described occurrence of scrotal cancer in workers with tar, paraffin and soot. One year later Manouvrier in Lyons (Bayet and Slosse, 1919) reported that in the workers with coal and its by-products frequently a syndrome was observed which he supposed to be caused by a poison and which he named " l'intoxication houillière " ; but he was not able to find out the responsible poison.

Pye-Smith in 1913 brought attention to the fact that pit-coal contains always arsenicated iron-pyrites in various proportion, and that its derivatives, such as soot, tar and pitch, contain still more of this poison. He pointed out that the affections of the skin often seen in these workers (keratosis, melanosis, warts and epithelioma) might be explained in that way, including the cancer of chimney-sweeps, much more frequently found in England than on the Continent, where wood coal was more commonly used.

Bayet and Slosse resumed these thoughts in 1919 with much emphasis. They found arsenic not only in pit-coal and its by-products, but also in blood, hair and urine of the majority of the workers engaged in making briquettes from pitch. They suggested that the clinical picture of the " l'intoxication houillière " is identical with that of chronic arsenicism ; melanosis, hyperkeratoses, troubles of the digestive tract, bronchitis, ulceration of the nasal fossa and the occasional occurrence of cutaneous cancer with special features ; appearance at a relatively early age, development on a keratosis (" verrue du brai "), frequent multiplicity, frequent localization on the scrotum and its neighbourhood. Bayet asserted that 70 per cent of the cases of both arsenical cancer and coal cancer had this location. So Bayet and Slosse concluded : " Le cancer arsenical et le cancer du goudron sont identiques."

This view, although accepted by some authors, as La Rossa (1925), has been rejected by both German and English authorities :

1. Some kinds of coal and tar, although free or nearly free of arsenic, cause also cancer in mice (Moeller, 1923 ; Daels, 1923 ; and Ross, *cit.* Bayet, 1930).

2. Bayet and Slosse (1919) themselves mentioned that their theory is unable to explain the fact that the workers handling coal itself (les " houilleurs ") are mostly not affected in contrast to the relatively high

incidence in workers employed with the by-products, such as tar, soot, pitch, etc. (Pye-Smith, 1913*b*).

3. The identity of the clinical pictures has been denied. The keratoses of the palms and soles so characteristic of chronic arsenicism are not typical of cancer caused by the by-products of coal. It is true that pigmentation is found in both conditions, but in pitchworkers it affects mostly the bare parts of the body. But the main objection is that the anatomical distribution is essentially different in pitch cancer and in real arsenical cancer, as Kennaway (1925) has pointed out in a special study. In contrast to the well-known predilection of arsenical cancer of fingers and toes, only two out of 209 cancers were situated on the fingers and none on the toes. Nearly one-half of the arsenical cancers but only 4 per cent of the pitch and tar cancers were situated on the fingers, hands, legs and trunk ; on the other hand 83 per cent of the pitch and tar cancers but only 23 per cent of the arsenic cancers occurred on the head, neck and scrotum ; so the special liability of the scrotum as the site of the pitch and tar cancer is not seen in arsenical cancer. The statistics of Bayet and Slosse were distorted by their procedure to exclude *a priori* all localizations on the palms and soles. Besides, the multiplicity of tumours is much more pronounced in the arsenical cases—75 cancers in 38 cases (the two cases of multiple cancers excluded), whereas in 123 persons with tar and pitch cancer only 144 tumours were recorded.

4. Finally, the theory of Bayet and Slosse has lost any basis by the definite identification of the real carcinogenic components of tar and pitch by the work of Kennaway and Cook.

So the theory of Bayet and Slosse has to be abandoned.

4. *Cancer of the bladder in aniline workers.*

Bayet and Slosse (1919), Bayet (1930) and Hamilton (1921) have suggested that the papillomatous and cancerous tumours of the bladder observed repeatedly in workers with aniline dyes may be caused by arsenic. They based this contention on the presence of arsenic as a contaminant in these dyes, the demonstration of arsenic in the urine (Wignall, 1920), and the possible similarity of cutaneous manifestations in these workers with arsenical dermatitis.

But this view was opposed by many authors (Posner, 1904, 1905 and 1924 ; Kennaway, 1925 ; Ullmann, Henry, 1930 ; Berenblum, 1932). Tumours of the urinary tract have been observed in arsenic poisoning but are very rare (see p. 206) ; the dye-workers suffering from cancer of the bladder present no signs of chronic arsenicism. Kennaway has pointed out that it is not clear why arsenic should cause cancer of the bladder in aniline workers, but cutaneous cancer in the general population. However, the aniline dyes are now free of arsenic, so that this poison can no longer be responsible for these bladder growths.

III. Comment on Occupational Arsenical Cancer.

In reviewing the observations in the literature in a critical way, a series of suspected occupational arsenical cancers must be excluded. There is no evidence whatsoever that arsenic is responsible for the pulmonary cancers of Schneeberg

and Joachimsthal, for the malignancies in coal and pitch workers, and for the bladder tumours in aniline workers. Only the occurrence in factories of sheep-dip and of arsenical insecticides, in gardeners, farmers and vintagers and a few incidences in miners and smelters (see above) really can be attributed to arsenic. The total number of such cases recorded until now is, as Hueper (1942) mentions, astonishingly small in comparison with the large number of persons exposed to arsenic in industry. It may amount to about 25 cases.*

The clinical picture of these occupational cancers is on the whole the same as in the more frequent and well-known medicinal cases. But there are some special features in the occupational cases (Fassrainer, 1936).

(1) All reported cases concerned males—a not surprising fact.

(2) The period of exposure to arsenicals is very long in most cases, ranging from 4 to 46 years, with an average of about 25 years (Table VI).

(3) Perforation of the nasal septum, a sign never mentioned in the therapeutic cases is very frequent as a concomitant in workers in sheep-dip factories (Legge, 1934), as well as in workers producing arsenical insecticides (Bridge, 1928, 1939). The reason why this symptom has not been observed in the medicinal cases may be found in the different route of exposure, the poison in the occupational cases being inhaled as dust and so irritating the upper air passages. But in the Reichenstein disease caused by drinking arsenic-contaminated water it is mentioned also as a frequent sign (see p. 216). It may be that such perforations occurred in some of the medicinal cases also, but escaped observation as they make no deformity and often no trouble, so that many of the affected are ignorant of this condition (Legge, 1934).

(4) Melanosis of the skin seems to be more frequent and more pronounced in the occupational cases. Whereas pigmentation is mentioned in only 16·4 per cent of the medicinal cases, it was present in at least 9 of 16 cases about which detailed notes are given, i.e. in 56 per cent (see Table VI). Legge (1934) seems to be inclined to ascribe this pigmentation of the skin to the working for years in contact with arsenical dust. But it may be remembered that in endemic arsenical cancer caused by drinking water (Reichenstein, Cordoba), pronounced melanosis is an almost constant sign.

(5) Keratosis, especially keratosis of the palms and soles, seems to be as frequent as in the therapeutic form. Among 16 cases described in detail keratoses are noted in 13 instances—81 per cent.

(6) The distribution of the lesions does not show marked predilection for the fingers, hands and feet as in the medicinal cases. This apparently is due to the fact that in the occupational cases the true Hutchinson type is rarer. In only three cases (2, 10 and 14) origin of the malignancy from a keratosis is stated. Most of the incidences belong to the multiple form frequently distributed all over the body. But only in two instances (cases 1 and 22) true multiple benign basal-celled growths seem to be present; in four cases (2, 5, 6 and 10) the biopsy showed squamous-cell cancers.

(7) So far as the predominance of the multiple type of epithelioma goes,

* To the 24 cases of Table VI may be added one case of Ayres and Anderson (1934); these authors mention one case of Bowen's disease and another case of epithelioma, but the latter may be identical with case 1 in Table VI. Besides, there are some doubtful cases reported, such as four from Henry (*cit.* Hueper, 1942) in workers exposed to an arsenic-containing powder. Hueper (1942) assumes a somewhat higher number of incidences (34), but it seems he has counted some cases twice over.

multiplicity seems more pronounced than in the therapeutic instances. But there are at least four cases with single growths (cases 1, 2, 5 and 6).

(8) Among the relatively small number of occupational arsenical epitheliomas four or five instances of pulmonary cancer are reported (cases 15, 16, 17, 18 and 20, the last perhaps identical with one of the former), whereas among the so numerous medicinal cases only two occurred. It is uncertain whether this is a mere coincidence, or if the irritation of the respiratory tract by arsenical dust is responsible. Unfortunately these cases are not reported fully; it is not mentioned whether melanosis and keratoses were present. A special committee was constituted to examine the problem (Merewether, 1943).*

The *treatment* of occupational arsenical cancer is the same as that in non-occupational cases. Of course the prophylaxis is here still more important. Therefore in many countries social-hygienic measures have been taken for the protection of the workers (Balthazard, 1930):

Prohibition of employment of women and young persons in factories and workshops in which arsenic is handled.

Notification of all cases of arsenical poisoning in factories and workshops in Great Britain by the Factories Act, 1937. Special notification of arsenical cancer seems not to be required (some other forms of cutaneous cancer, such as tar cancer, are notifiable—Statutory Rules and Orders, 1919, Vol. I, p. 710).

Preventive technical measures in the factories, such as avoidance of dust, use of dust-proof clothes and respirators, prohibition of meals taken in the work-rooms, provision of adequate facilities for washing, etc. (in Britain, Factory and Workshop Act, August 17, 1901, Part IV, 75; Factories Act, 1937, Part IV, 47 and 48).

Regular medical inspection of the factories and workshops. Workers with beginning arsenical keratosis or other signs of arsenicism are forbidden to continue their work, but have to receive compensation for loss of income.

The most simple and at the same time the most efficient way to deal with arsenical occupational cancer would be to abolish the general use of arsenic-containing material generally. That has been done partly by the prohibition of the use of arsenical colours in house painting, in wall papers, etc. As now nearly all occupational cases are caused by arsenical anti-parasitics and insecticides, the replacement of these by modern arsenic-free compounds such as DDT would settle the whole question.

D. ARSENICAL CANCER IN ANIMALS (EXCEPT EXPERIMENTAL CANCER).

The first mention of the occurrence of chronic arsenicism in animals is again by A. Paris in 1820. He reports that in the neighbourhood of the copper-smelting works of Cornwall and Wales horses and cows commonly lose their hoofs, and not infrequently suffer from cancerous affection of their rumps. This assertion, quoted over and over again, occasionally even with unfounded additions, has been strongly criticized by Kennaway (1942, 1943; see also Hueper, 1942*b*, 1943). He objects that no attempt was made to show that arsenic was the active substance, and that the carcinomatous nature of the alleged tumours never has been established. Besides, the story of Paris has not been confirmed

* The members of the Arsenic Committee of the Medical Research Council are Professor M. J. Stewart (Chairman), Professor A. Bradford Hill, Dr. Donald Hunter, Dr. S. A. Henry, Sir Ernest Kennaway, Dr. A. N. Currie and Dr. Joan Faulkner (Secretary).

by later reports from that area. So Paris's report should never again be quoted as an example of arsenical cancer in animals.

In the district of Freiberg (Saxony) smelting works for many decades affections of horned cattle are known as "Huettenrauchkrankheit" (smelting fume disease). The clinical symptoms consist of chronic diarrhoea, cough, scleroderma, chronic squamous eczema, paralytic weakness and emaciation. These symptoms led to the thought that they may be caused by chronic arsenic poisoning. This hypothesis was confirmed by the demonstration of arsenic in the organs of the diseased cattle (Haubner, 1878, *cit.* Prell 1936/37; Wobst, 1925; Hofmann, 1937, *cit.* Kennaway, 1942, 1943). But no case of cancer is mentioned in these observations.

Since 1930 extensive arsenical injuries have been observed among wild animals (red deer, roe deer, hares and foxes) in the Tharandt forest, also in the region of Freiberg. The disease, most marked in deer, is characterized by loss of hair, blackening of the underlying skin, thickening of the stratum corneum and extreme emaciation. In the preceding years many bee-swarms were lost, and the beekeepers attributed that to an intoxication by the smelting fumes of Halsbruecke and Muldenhuetten, 16 and 18 km. away. Prell (1936/37) was able to confirm this view by finding in the pollen up to 8·8 mg. per cent arsenic, in the dead bees up to 0·0011 mg. per bee (= 1·3–2·6 mg. per cent), the lethal dose for a bee being 0·0001–0·0002 mg. So he concluded that the beekeepers were right, and supposed that the disease of deer was due to the same cause. He emphasized the similarity of the symptoms with those of arsenical poisoning in man, drawing attention to the horny warts sometimes scattered over the skin. Besides, he often found a considerable amount of arsenic in the stomach (e.g. 47 mg. per cent in a hare), in the hair (up to 1·2 mg. per cent), less in the liver; sometimes no arsenic could be detected. Prell says nothing about occurrence of real epitheliomas. But Hofmann (1937, *cit.* Kennaway, 1942) discovered in the course of his investigations one case of cancer in a roe. Kennaway (1942), in his criticism of Hueper's view, pointed out that this single case among 143 deer and 27 hares exposed to arsenic gives, of course, no basis for the occurrence of arsenical cancer in animals.

A high mortality of bees and fish was also reported from Reichenstein (Kathe, 1937).

Nieberle (1939) described the occurrence of endemic carcinoma of the nasal mucosa in a highly inbred flock of Hampshire sheep in the Limbach estate in the remoter neighbourhood of the smelting works again of Freiberg in Saxony. Although in the tumours, in the brain and in the liver only extremely minute amounts of arsenic (0·01 mg. per cent) were found, Nieberle thinks that "a certain connection of this disease with the locality and with an injury by arsenic can scarcely be excluded." This cautious conclusion does not support Hueper's assertion that an environmental type of arsenical carcinoma has been observed in animals (Hueper, 1942; Kennaway, 1942).

E. EXPERIMENTAL ARSENICAL CANCER.

It has been tried to prove the carcinogenic property of arsenic by experiments in animals—especially after the successful experiments with tar and its constituents. Different animals were used and different ways of application.

Local Application on the Skin.

Leitch and Kennaway (1922) painted a solution of potassium arsenite in alcohol (1·8 per cent, later 0·12 per cent) three times daily on the skin of 100 white mice through a long period. Most of the mice died within three months. In one of the survivers after 86 days a tiny wart appeared on the spot of application, after 98 days a second. The wart developed into a metastasizing squamous-cell epithelioma. In a second series (Leitch, 1923) not a single positive result was obtained. It is difficult to explain why only a single experiment was successful. Spontaneous epitheliomas of the skin are very rare in mice, and the localization on the very spot of application seems to prove that the cancer in this case was caused by the administration of arsenic. The fact that no more positive results were obtained may partly be explained by the difficulty in keeping the animals alive for a sufficient time. Probably there must be—besides the administration of arsenic—some other unknown local or general factors influencing the success of such experiments. Lipschuetz (1924), repeating these experiments of painting with an alcoholic solution of arsenious trioxide, recorded only hyperkeratoses and pigmentations but no malignancy. Raposo (1928) painted the inner surface of the ears of rabbits with a mixture of 10 per cent arsenic trioxide and vaseline. Besides hyperkeratoses and papillomas in several cases he obtained a cancroid in one case. It is not reported whether controls with vaseline alone were performed.

Oral Application of Arsenic.

Leitch and Kennaway (1922) obtained negative results in rats and mice, Maisin (1934) in mice. Cholewa (1934), feeding 10 mice with arsenic for one year (0·05–0·1 mg. daily), observed in two of the animals blastomas of the lungs ; in one rabbit a sarcoma of the ear after 6 months. In the experiments of Finner and Calvery (1939) with dogs and rats fed with arsenious trioxide and with arsenates, pathological changes developed but no growth is reported. Hueper (1942*a*) experimented with congenitally hairless rats ; in a series of 10 animals he obtained in one rat surviving for 21 months a cancer of tricho-epitheliomatous structure. Nothing is said about the frequence of spontaneous cancers in these animals ; it is only stated that they develop normally papillary warts in their hyperkeratotic skin (that was the reason why Hueper used them for his experiments).

Parenteral Application of Arsenicals.

Bierich (1922), as well as Moeller (1923) and Lipschuetz (1924), injecting white mice hypodermically with Fowler's solution, had negative results. Askanazy and Girod (1926) injected rats with arsenious acid ; in one of these animals after some months an osteoid sarcoma developed from the follicle of a cysticercus. White (1927) reported five negative experiments in chickens. Schinz (1942) made deposits of (? metallic) arsenic by injecting it in the thighs of rabbits. In one animal after six years a pulmonary giant-cell sarcoma developed. Spontaneous malignancies are said to be quite rare in rabbits. Ebert (1929) observed, after intradermal application of a small dose of arsphenamine given to a man of 63, microscopical changes which he describes as " precancerous," similar to Bowen's disease.

As the experiments with arsenic alone did not give definite results, other ways were tried.

Puccinelli (1930*a*) found very little influence of arsenic on the appearance of spontaneous primary lung tumours in mice.

Many authors studied the effects of arsenicals on pre-existent tumours in animals. The results were widely divergent. Some investigators, as Sticker (1911), Funk (1915), Bierich (1922), Moeller (1923), Rosen (*cit.* Schiller, 1926), and Schiller (1926) found no effect. Montemartini (1928) reported an inhibitory effect of intravenous injections on transplanted cancers in mice.

Others observed a stimulating effect—Antonoff (1928) on Flexner-Jobling rat carcinoma, Califano (1931) on Teutschlaenders strain in chickens; Hueper and Itami (1933) think that intravenous injections of neoarsphenamine were leading toward increased malignancy of spontaneous tumours in mice. Dustin and Gregoire (1933) experimented with mice bearing sarcomas type Crocker; as soon as 9 hours after injection of sodium cacodylate they observed a " veritable explosion caryocinétique " in the transplanted tumoûr (see p. 231).

Other investigators studied the influence of arsenic on the development of experimental tar cancer. Schiller (1926) reported that arsenic prevented the precancerous changes in the skin of tarred mice from developing in carcinoma; but it did not influence a fully established tar cancer. Puccinelli (1930*a*) found a slight delaying action of arsenic trioxide on the appearance and growth of tar cancer in white mice. On the other hand, Ciechanowski, Morozowa and Wilhelmi (1925) observed a distinctly accelerating effect of oral ingestion of potassium arsenite on the development of tar carcinoma in rabbits.

Following the theory that arsenic may be favourable to the development of growth from embryonic cells, other investigators combined the ingestion of arsenicals with inoculation or implantation of embryonic cell material.

Carrel (1925) started from the observation that the filtrable agent of the Rous sarcoma is able to transform *in vitro* macrophages into sarcoma cells. Supposing that this filtrable agent is produced by the cells through the influence of a non-specific chemical substance, he injected 16 chickens with a mixture of embryonic chicken pulp and arsenious acid. In four of these fowls after a few days fatal fusicellular sarcomas developed. The tumours contained a filtrable agent resembling a virus. Carrel suggested that the arsenious acid had caused the formation by the tissues of this agent.

These sensational experiments were repeated by many investigators; but only some of them had similar results.

Fischer (1926) reported such a similar result in a chicken which was inoculated with embryonic spleen tissue cultivated in a medium containing a very small quantity of arsenic trioxide. White (1927) confirmed Carrel's results; the sarcomas arose from the embryonic tissue, not from the tissues of the host. Haagen (1928) also repeated Carrel's experiments, with a positive result; in addition he injected normal monocytes from chickens together with very dilute solutions of arsenious acid in 24 fowls; after three weeks rapidly growing metastasizing and transplantable sarcomas of the Rous type developed in 16 of the animals. Petroff and Krotkina (1928) injected 97 rats with embryonic pulp together with a suspension of arsenious acid; in three of the rats six months later sarcomas appeared. It seemed improbable that these were spontaneous tumours as they arose on the spots of injection. On the other hand the authors

reported the development of sarcomas after injection of embryonic pulp alone; so the aetiological role of arsenic in these experiments is not clear (spontaneous tumours were very rare—only one in 300 rats). Krotkina (1934) observed metastasizing transplantable growths after injection of embryonic pulp in a rat previously treated with arsenic.

But all the other authors repeating Carrel's experiments were not able to confirm his results:

Pentimilli (1926), Deelmann (1928), Maisin and Dupuis (1929), Kauffmann (1929), Felloni (1930), Laclau and Pillado-Matheu (1930) and Lapidaria. Only small embryomas (teratomata) occurred sometimes but no cancers.

Negative results in rats and mice were obtained by Collier and Hartnack (1929), Puccinelli (1930*a*, *b*), McJunkin and Cirkit (1929). McJunkin and Cirkit succeeded only in one out of 50 rats in getting a cystadenoma suggesting malignancy.

Some authors, such as Deelman (1928), Maisin and Dupuis (1929) and Begg and Cramer (1929), suggest that Carrel's results were possibly due to minute contamination with traces of Rous' sarcoma virus, not to the presence of arsenic.

Askanazy (1926) examined the influence of arsenic on the growth of embryonic tissues (stomach, intestine) transplanted into the stomach of rats receiving a small quantity of Fowler's solution with the drinking water. After 11 months a fusicellular metastasizing sarcoma of the stomach developed in one out of nine rats; in two others an adenocystoma appeared after 13 and 14 months respectively, also on the spot of implantation; whereas in 18 controls without arsenic the transplants disappeared except in one case in which a small sarcoma of the liver arose. Askanazy pointed out that for the genesis of blastomas the presence of several factors may be necessary: besides a general constitutional predisposition ("allgemeine Koerperbereitschaft") and a local predisposition (represented in these experiments by the embryonic tissue), a third factor, an irritant of oligo-dynamic efficiency, e.g. arsenic.

Fischer-Wasels (1928, 1929) and his co-worker Buengeler (1930) combined two supposed carcinogenic factors in a different way. They produced chronic arsenicism by feeding or injecting rabbits or mice with potassium arsenite for many months; then they injected scarlet red oil into the skin or (in female mice) into the breast, or they made local burns to induce local regenerative processes. Among 18 rabbits, in one a cancer of the skin developed; and in 5 mice (= 25 per cent) adenocarcinomas of the breast, all controls remaining negative. Spontaneous cancers of the breast were rare in these mice (only 4 cases among 2000 animals). The authors' interpretation was that chronic arsenic poisoning had produced a general pre-disposition for cancer after a sensitizing period of some months, the local procedure a local anlage. Further they found out that the general disposition for growth in these experiments with arsenic was characterized by certain changes of the metabolism of the tissues, proved by Warburg's methods: decrease of oxygen consumption (checking of the respiration), increase of anaerobic formation of lactic acid, and by aerobic glucolysis.

Summarizing the experiments on animals it can be stated that arsenic alone has given only doubtful results. Difficulties were caused by the occurrence of spontaneous tumours in the animals, by the high toxicity of arsenic leading to an early death of the animals, and by the fact—well known from clinical ex-

perience—that arsenic has a very long latent period for the production of malignancy, usually surpassing that of the average life of the animals. The combined experiments—arsenic plus embryonic cells or arsenic plus local influences on the tissues—have proved more successful. No critical objections to these experiments seem to have been put forward so far.

F. PATHOGENESIS OF ARSENICAL CANCER.

Even now a really satisfactory theory of the pathogenesis of arsenical cancer does not exist, although many clinical observations and much experimental work are available.

It is a fact that arsenical malignancy is very often preceded by other manifestations of arsenical poisoning, especially keratoses and pigmentation. Therefore, arsenical cancer might be conceived as a " second disease " in the sense Roessle used this term.

Stelwagon, Gilchrist (*cit.* Hueper, 1942*b*), Dubreuilh (1910) and de Silva (1918) held the opinion that arsenic is not directly responsible for the cancer, but only for the development of the keratoses. " C'est l'arsenic qui fait la kératose, mais la kératose évolue ensuite spontanément vers le cancer " (Dubreuilh). This view was supported by the fact that keratoses of different origin sometimes undergo malignant transformation as well, e.g. keratoses due to X-rays, radium, irradiation, senile keratoses and leukoplakia oris.

This view was opposed by Schamberg (1907*a*): " The mere fact that there is an intervening hyperkeratosis does not relieve arsenic from the responsibility of the production of the malign disease." He stressed the fact that the vast majority of keratoses (congenital keratoses, tylosis, clavi, etc.) are not in the least inclined to become malignant. Besides, this keratosis hypothesis is insufficient, because it is now well established that many arsenical growths arise in previously normal skin.

Stewart (1934, *cit.* Hueper, 1942*b*) suggests that the pigmentary disturbances set up by arsenic may be related to the development of malignancy. It has been referred to that melanin contains cyclic hydrocarbons which might be carcinogenic. But in the majority of cases of arsenical cancer abnormal pigmentation is missing.

An explanation of the carcinogenic property of arsenic has to start from the pharmacological qualities of that poison; unfortunately they are still far from being clear.

It is known that arsenic has local as well as—after its resorption—general effects.

Local effects of arsenic.

Arsenic working as a *local irritant* in high concentrations causes necrosis. That local irritation can play a role in the causation of malignancy is generally accepted. Further, it is certain that in some cases of chronic arsenicism, especially in occupational cases, signs of external irritation occur: eczema of hands, conjunctivitis, perforation of the nasal septum, etc. But the usual arsenical cancer of the skin cannot be caused by such a direct irritation; for it does not appear at the sites of application. If that be the case, the cancer—

at least in the medicinal cases—would be expected in the mouth, oesophagus, stomach and in the intestines; frequent cancer of the jaws would be expected after the common use of arsenic by dentists, but not localization on the skin. Besides, the nearly always negative result of external experimental application of arsenic speaks also against local irritation as the main etiological factor.

So arsenical cancer differs essentially from other kinds of malignancy, such as tar cancer, X-ray cancer, radium cancer, in which the main effect is seen at the site of application (Semon, 1922; Guggenheim, 1933,). But it must be remembered that Murphy and Sturm, in mice subjected to painting with tar, observed an increased incidence of pulmonary cancer, and that similar results were reported by Schabad (1935) and Cook, Haslewood, Hewett, Hieger, Kennaway and Mayneord (1937). That seems to show that tar, besides its well-known local action, has also a carcinogenic property after its resorption.

Only a few observations were advanced by some authors as an indication that local external irritation may occasionally play a part in arsenical malignancy. It was suggested that in some occupational incidences malignancy arose on the place of application (Ullmann, 1930; Fassrainer, 1936; Aitken, 1944). Eggers (1932) quotes especially the cases of sheep-dip cancer as caused by constant irritation of the skin by arsenical dust; but the distribution of the lesions in these cases was mainly the same as in medicinal multiple superficial epitheliomas (see p. 223). The occurrence of pulmonary cancer in workers exposed to inhalation of arsenical dust was also attributed to local irritation, but without real proof. Besides, the occasional occurrence of local "pre-cancerous" changes after injection of arsphenamine (Ebert, 1929) may be mentioned, and the very rare positive experiments with external application in animals (see p. 226).

General Pharmacological Effects of Arsenic.

Hermann (1879), Schmiedeberg (1906) and Heubner (1907) suggested a paralysis of the capillaries as the fundamental general pharmacological effect of arsenic after its resorption. They attributed to it the frequent inflammations of the mucous membranes and of the skin. But it is not clear in which way such poisoning of the capillaries could lead to malignancy. Besides, the view of these authors was opposed by others (Heffter and Keeser, 1927).

Ringer and Murrel (1878/79) declared arsenic to be a poison to all nitrogenous tissues. And since then many investigators held the view that the basic effect of arsenic is its *action on the metabolism* of the cells.

Binz and Schulz (1879) suggested that the action of arsenic is connected with the mutual transformation which arsenious acid (As_2O_3) and arsenic acid (As_2O_5) undergo in the presence of living protoplasm; they pointed out that the effects of arsenic result from the development of active oxygen originating in these processes, stimulating the cells eventually to exhaustion and death. Brooke and Roberts (1901) supported this *oxidation hypothesis* as the best explanation of the facts. But it seems that the supposed increase of cellular oxidative processes has not sufficient experimental basis. Even the famous basal experiments of Binz and Schulz have been criticized; the reduction of As_2O_5 has been proved, but not the oxidation of As_2O_3 (see Heffter and Keeser, 1927).

In contrast to the oxidation theory, it is known for a long time that arsenic in very small quantities checks the processes of oxidation and reduction in the body (Onaka, 1911). That may result in an increase of body weight and body fat, often aspired to and achieved in the therapeutic use of the drug; increase of body protein and bone tissue was observed too (Weiske, 1875).

Barry, Bunbury and Kennaway (1928) demonstrated *in vitro* such a retarding action of arsenic upon three different oxidation-reduction processes, namely: hypoxanthine-xanthine oxidase of rat or mouse-skin, oxidation of acetaldehyde with colloidal platinum, and the system propylaldehyde (or acetaldehyde), glycerine and phosphate. The authors themselves emphasize that one cannot with any certainty compare reactions in the test-tube with changes in the human skin, which require months and years. But they are in accord not only with the just-mentioned results of Onaka and similar results of Dresel (1926 and 1928), Banga, Schneider and Szent Gyeorgyi (1931), and of Victorisz (1931) and others, but also with the above-mentioned work of Fischer-Wasels (1928 and 1929) and Buengeler (1930), who found in animals predisposed to cancer by ingestion of arsenic a decrease of oxygen consumption and increase of anaerobic formation of lactic acid.

Barry, Bunbury and Kennaway express the idea that arsenic may induce cancer not by any direct action, but by causing accumulation in the tissues of some (carcinogenic) organic compounds which would otherwise be oxidized or reduced to other forms.

Bunsen had qualified arsenic as a "protoplasmic irritant." Many authors stick to the view that it acts as an "auxetic," by *direct chemical stimulation* of the epidermal cells leading to their proliferation and "cancerization" (Bland-Sutton, 1916; Osborne, 1925; Ullmann, 1930). Dustin and Piton (1925, 1929) and Piton (1929) observed after injection of arsenicals (especially cacodylate of soda) in mice numerous mitoses in the thymus, in the lymph-nodes and in the Lieberkuehn glands—"action caryocinétique." Rocmans (1930) attributed this effect to an alkalosis, but this view was criticized by Dustin (1930). On the other hand Throne and Myers (1935) have found in some patients with arsenic retention and (non-malignant) lesions of the skin a rise in the CO_2 content of the blood suggesting an alkalosis.

Ullmann (*cit.* Mayer, 1933, and Throne and Myers, 1935) reported at the International Congress of Dermatology, 1930, that a solution of one part of arsenic in 40 millions of water, i.e. 0·0025 mg. per cent, added to embryonic cells acts on them in a tumour-forming manner; he asserts that this action on the cells of the hair sheaths and walls of sweat glands explains the development of arsenical cancer.

To explain the so well marked *predilection* of arsenical cancer *for the skin* one has to remember that arsenic has a special affinity for structures of ectodermal origin. After ingestion it accumulates in the epidermis, sweat and sebaceous glands and their ducts, hair follicles and hairs. Both Bruenauer (1921) and Osborne (1925) were able to identify it there with the histochemical method. Some authors ascribe this affinity to a special relation of arsenic to the keratin containing large amounts of sulphur (cysteine, etc.).* So it may be understood

* See Labes, 1929, and Rosenthal and Voegtlin, 1930.

that arsenic acts in these places as an irritant, causing keratinization and possibly malignancy.

Certain authors asserted that the keratoses originate from the orifices of the sweat glands (Ullman, 1930; Bruenauer, 1921), and suggest that this fact is connected with the excretion of arsenic in the perspiration. But this view is not generally confirmed (Brooke and Roberts, 1901).

One of the difficulties in understanding the pathogenesis is the often very *prolonged interval* between cessation of the poison and starting of the malignancy (see p. 210). Some authors doubt whether after such a long time arsenic can still be present and act in the skin. Ullmann (*cit.* Hueper, 1942*b*, p. 54) suggests that the cells are "cancerized" during the exposure to arsenic, but remain quiescent for a long time. But the example of argyrosis shows, as Franseen and Taylor (1934) remark, that a drug can remain in the skin for a lifetime; and the presence of arsenic in the skin after a very long time was demonstrated by chemical and histochemical methods (Montgomery, 1935).

Another fact to be explained by the theory is that arsenic produces epitheliomas *only in very few* of the many persons exposed to that poison. Many thousands of patients are receiving this drug, but in comparatively few arsenical keratoses develop, and in still fewer epitheliomas (Wile, 1912; Sulzberger, discussion to Bloom, 1936; Satenstein, *ibid.*). And in the positive cases the dose of arsenic as well as the duration of the latent period and the type of epithelioma show very great differences. Ebert (1929) observed precancerous changes after injection of arsphenamine only in certain persons. And in analogy the attempts to produce experimental cancer with arsenic in animals were successful only exceptionally.

All these facts suggest that in "arsenical cancer" *arsenic is not the only aetiological factor*; others must be present too. On this point all authors seem to agree (Schamberg, 1907; Wile, 1912; de Silva, 1918; Askanazy, 1926; Buengeler, 1930; Milch, 1932; Frost, discussion to Ayres, 1934).

Some observations suggest that the *individual reactivity* to arsenic shows a marked variability (Schondorf, *cit.* Hueper, 1942, Throne and Myers, 1935). Throne and Myers have suggested that these differences may be related to variations in the excretion of the poison. Cornbleet (discussion to Montgomery, 1941) found that patients showing arsenical keratoses take a longer time to excrete a given amount of arsenic than control patients; he concluded that in such patients the drug is retained in the epidermis for protracted periods so as to irritate and to produce keratoses and cancer.

Other authors suggest a predisposing local abnormality of the skin, perhaps a *local sensitivity* (idiosyncrasy) to arsenic analogous to contact dermatitis. Investigations with patch tests (Sulzberger, discussion to Bloom, 1936) had no conclusive results.

Another fact suggesting a special sensitivity of certain individuals is the high frequency of psoriatics among the patients with medicinal arsenical cancer (see p. 194).

The role of *heredity* as a predisposing factor has been mentioned by several authors. It is well known that hereditary predisposition is very important in certain kinds of clinical as well as experimental malignancy. It is noteworthy that in psoriasis too a hereditary factor is well established (Case 130 in Table I belongs to a psoriatic family). It is true that in only a few cases of arsenical

TABLE I.

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
1	White (1885), "case 1"; Hutchinson (1887, 1888) case Dr. W. (1895, 1898, 1903) (picture)	M. 44	S. Fow., S. Don.; large quant. beg. 20 y. ago f. 6 y.	Ker. p. and fingers (corns) beg. 10 y. ago f. 6 y.	One: r. p. on ker. beg. 1–2 y. ago. One: betw. l. fore- and middle finger on ker.	Metas. Death
2	White (1885), "case 2"	M. 52	As. apparently many y.	Ker. p.	One: p. suff. r. wrist on ker. 10 y. One: betw. fingers on ker. Three: nr. anus, upon penis, groin	Carc. Death.
3	v. Hebra (1887), *cit.* Pye-Smith (1913)	M. ? 50	As. Psor. (probably)	Over 100 "warts" on the body	One: r. arm. One: r. thigh, and other parts on warts	Carc. Death.
4	Hutchinson (and Tay) (1887, 1888)	M. 34	As. long time. Psor.	Ker. p. and s, feet s. 1½ y.	One: scrot., some months preceded by a wart	..
5	Hutchinson (and Chiene) (1887, 1888)	M. 55	As. many y. Psor.	Ker. p. and s. (corns)	One: l. s. One: toe on ker. Later: carc. of stomach	Death.
6	Hutchinson (and Allbutt) (1887, 1888)	F. 25	As. repeatedly. Pemphigus	Roughness of p.	One: nr. crista ilei on spot of rough skin	Metas.
7	Lane, W. A. (1894); see also *A. D. & S., Chic.*, **131**, 46	M. 60	S. Fow. f. 30 y. Psor.	Not stated	11 growths: 4 ulc. l. forearm in healthy skin 7 ulc. scrotum and perineum	Ep.
8	Hutchinson, J. (1894)	M. 35	As. 20 bottles, 10 y. prev., f. some months. Acne	Ker. p.	One: hand, on ker., 10 y. after stopping As.; 6 y. later two scrotum	Carc.
9	Hutchinson, J. (and Bullock) (1898, 1903) (picture)	M. 46	As. liberally f. many y. Psor.	Ker. s., legs and elsewhere	One: above pubes. One: back (s. 3 y.)	Metas. Death.
10	Hutchinson, J. (1898)	F. 45	As. f. 20 months. Epilepsy	Ker. p. and s. some y. after taking as.	One: r. hand on ker. One: below l. breast	Metas. Death.
11	Hartzell and Stellwagon (1898, 1899) (picture); Stellwagon (1910 ?)	F. 35	As. f. long period. Psor.	Ker. p. and s. 9 y. aft. beg. of psor.	Num. small superf. ulc. on ker. of hands and fingers, consid. time after beg. of ker. Two: l. heel One: over l. breast One: l. groin	Carc.
12	White, J. C. (1899)	M.	As. Psor.	Ker. p. and s.	One: gr. toe, on ker.	..
13	Hyde (1899)	M.	As. Psor.	Ker. p. and s.	One: middle finger, app. on ker.	Metas.
14	Ullmann (1898, 1900) (picture) (1917)	F. 37	S. Fow. f. 6–7 y. Acne	Ker. p. and s. Pig.	One: forehead, in ker. One: l. heel One: r. ankle	Bas. c. ep. Sq. c. ep.
15	Crocker and Pernet (1901)	M. 60	As. 40 y. ago, f. 2 y. Psor.	Warty specks, hands	One: r. hand, uln. border on a speck, 2 y. ago Two others in neighbourhood	Sq. c. ep. Excision. Rec.
16	Hutchinson, J. (1902, 1903) (plate)	M. 70	As. f. long periods. Psor. (?)	Ker. p. and s.	Soon after ker. One: r. forefinger on ker. One: back of r. shoulder	Carc.
17	Darier (1902), *cit.* Pye-Smith (1913*b*) "case 22"	M. 47	As. large quant. Chr. bronchit.	Ker. p. and s. (corns). Pig.	Three: hands on ker. One: neck One: r. upp. eyelid	..

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
18	Brocq (1902), *cit.* Schamberg (1907)	M. 35	As. large quant. 15 y. (ab. 10 g. As_2O_3. Chr. bronchit.	Ker. p. and s., warty pig.	Two: r. p. on ker. Two: l. p. One: neck	Carc.
19	Hutchinson, Browne and Blackstone (1903) "case 4"	M. 62	As. f. many y.	Ker. p. and s.	One: buttock One: face	Carc.
20	Schamberg (1907), "case 1"	M. 62	S. Fow. *ca.* 30 drops daily f. 3 months 25 y. Psor.	Ker. p. and s., warty pig.	One: dors. r. thumb on ker.	Carc.
21	Hartzell*	M. 55	As. Psor.	Ker. p. and s.	One: r. p. on ker.	Amputat. Rec. Death.
22	Dubreuilh (1910), "case 3"	F. old	As. large dos. Pemphigus	All signs of chron. arsenicism	Carc. mammae	..
23	Dubreuilh (1910), "case 4"	M. 74	S. Fow. large quant. 15–20 drops 20 y. Psor.	Ker. p. and s. s. ab. 15 y.	One: l. thumb on ker. One: r. middle finger 2 y. Sev. les. trunk	Sq. c. ep.
24	Gray (1912)	F. 56	As. 32 y.	No ker.	One: nr. glut. cleft, continuous with a patch of psoriasis 10 y.	Bas. c. ep.
25	Loewenberg (1912) "case 1," see also disc. to Ullmann (1922)	M. 50	As. Psor.	Ker. p. and s., backs of hands and feet, warty	One: back l. hand on a ker. papillomatous, a. others on. One: flex-side of forearm, in normal skin	Sq. c. carc. Amputat. Rec. Sq. c. ep.
26	Loewenberg (1912) "case 2"	M. 50	As. Psor.	Ker. p., warty	One: r. p. 1½ y. from a ker.	Death on interc. di
27	Wile (1912) (plate)	M. 29	As. large quant. 10 y. ago f. 2 y. f. complexion	Ker. hands and feet diffuse and punct. 4 y. after starting As. No pig.	One: l. ring-finger on ker.	Carc. Metas.
28	Schamberg, J. F. (Philad. Derm. Soc., *cit.* Pye-Smith, 1913*b*), "case 28"	M. 43	As. large quant.	Ker. trunk. Telangiect.	One: r. nipple. Num. epith. all over the trunk	..
29	Nutt, Beattie, and Pye-Smith (1913 *a* and *b*)	F. 29	As. f. 7 y., begin. 22 y. ago. Psor.	Ker. p. and s. (corns) 1½ y. under wedding ring Ker. on the whole body	One: l. ring finger on ker. 10 months. One: l. middle finger One: pubes on ker. Three: vulva, strawberry-like, s. 1½ y.	Sq. c. ca Amputat. Rec. Death.
30	Fordyce-McKee (1914)	M. 37	S. Fow. f. long per. Psor.	Ker. p. Pig.	14 les. back, arms, leg	Bas. c. ca
31	Trimble (disc. to Fordyce-McKee, 1914)	M. 65	As. f. long per. Psor.	Ker.	One carc.	..
32	Bland-Sutton (1916)	F. 60	S. Fow. more or less contin. f. 30 y. Psor.	Nothing noted	One: below l. knee in patch of Psor. 3 y.	..
33	Ullmann, K. (1917, 1932), "case 2"	F. 33	S. Fow. 15–17 y. ago f. 1–2 y. Chlorosis and complexion	Ker. p. and s., warty, mult. ker. Leukoplakia oris. No pig.	One: heel, 1 y. ago One: index, ½ y. ago Later: one tongue Carc. of mucosa oris on leukoplakia	Sq. c. ca pagetoid Death.

* Discussion to Schamberg (1907). See also Pye-Smith (1913*b*), "case 25."

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
34	Ullmann, K. (1917), " case 3 "	M.	S. Fow. f. 2 y. M. mac. Werlhof	Acute ars. pois. 1 y. later; ker. p. General melan.	One: heel, in ker.	Death (tub.)
35	Stillians, A. W. (1919)	M. 57	As., large dos. f. 1 y., 30 y. ago, f. prevent. malaria	Ker. s. 25 y.	One: l. p. 20 months	..
36	Pusey (disc. to Stillians, 1919)	..	As. Psor.	Ker. hands	One: hand, in ker.	..
37	Wise, F. (1920)	M. 42	S. Fow. 20 y. ago f. 6 weeks. Pemphigus	Ker. p., sacr. and lumb. reg.	One: sacr. reg. in ker.	Sq. c. carc.
38	Hamilton, G. R. (1921)	F. 46	S. Fow. s. 36 y. 9 months in a year. Psor.	Ker. p. and s., back of hds. (corns) s. 20 y.	One: r. arm not in a patch of psor.	Sq. c. carc. (mal. papillom.)
39	Alexander, A. (1921)	M. 35	S. Fow. 15 y. w. interrupt. Psor.	Ker. p. (not s.) s. 137. Pig. s. 13 y.	One: r. thigh on a patch of psor.	Bas. c. carc.
40	Ullmann, K. (1922)	..	S. Fow. sev. y.	Ker. (p. and s. ?)	One: toe One: leg	Death.
41	Ullmann, K. (1922)	..	S. Fow. sev. y.	Ker. (p. and s. ?)	One: toe One: finger	Death.
42	Semon, H. C. (1922) (picture)	M. 40	As. f. 7 y. Psor.	Ker. p. and s. (warty) after 7 y. Pig.	One: r. sole in ker. 14 y. after stopping As.	Sq. c. carc. Amputat.
43	Nander (1923)	M. 65	Pil. asiat. 2 g. in 1 y. Psor.	Ker. hands and feet. Pig.	Mult. ulc. both shins in psor. spots Forehead: non-malig. adenomata	Sq. c. carc.
44	Oliver (1923)	M. 42	As. over a long period. Acne	Ker. p. and backs of the hands	4 or 5 ep. on the body	..
45	Haxthausen, H. (1923)	M. 65	As. pills (prob.) 3 *t.d.* during 9 y. beg. ab. 22 y. ago " for the nerves "	Num. ker. trunk and extr.; no ker. p. and s.; begin. 4–6 y. after stopp. the pills	Mult. (20) carc. spread over the trunk, one in l. ax. ulc.	..
46	Aliferis (1924, " case 1 "	M. 47	S. Fow. and As. pill. Psor.	Ker.	One: r. knee One: nr. crena ani (not certain whether on patch of psor. or on ker.)	Sq. c. carc.
47	Aliferis (1924), " case 2 "	M. 67	S. Fow. and As. pills sometimes in 33 y. Lichen rub. planus	Ker. Hyperker. sub ungualis. Erythrodermia	Five, arising from ker., breast, back	Carc. death.
48	Barber, H. W. (1925)	M. 70	S. Fow. 6 drops 3 *t.d.* Psor.	Ker. p. and s. Pig. Chr. arsenical dermatitis	Three (?) fungating tumours: scalp, face, ears Two flat growths upper arms	..
49	Schwartz (1926)	M. 35	Medicine cont. As. s. 8 y. (?). Dermatitis herpetif.	Ker. p. and s., fingers. Hyperhidros. General keratos. Pig.	One: r. thumb, palmar surf. der. from ker. les. besides groups of papules and vesicles on trunk; some resemble Bowen d.	Sq. c. carc
50	Levin, O. L. (1926), " case 1 " (see also Anderson, 1932)	M. 45	Arsphenamine As. p. os and inject. sev. y. Syphilis. Psor.	Ker. p. and s., trunk	One: l. chest One: l. arm One: neck	Bas. c. carc. Bas. c. carc.
51	Levin, O. L. (1926), " case 2 " (see also Fraser, 1929)	M. 35	As. inject. and asiatic pills. Dermatitis herpetif. ?	Ker. p. and s., buccal muc.	One: l. thumb	Sq. c. carc. Metas.
52	Herxheimer (1939)	M.	As. f. 10 y. Psor.	Nothing noted	Sev., trunk	..

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
53	Harbitz, H. F. (1927)	F. 46	Arsphenamine inj. 15 y. ago. Syphilis	..	Fibrosarcoma on spot of injection (left intersc. reg.) 12 y. after inj.	..
54	Oliver and Finnerud (1928)	..	S. Fow. beg. 22 y. ago f. 11 y. Acne	Ker. p. (warts) 5 y. after starting As.	One: lumbo-sacr. reg. One: l. thigh	..
55	Fraser (1928), "case 1"	M. 59	As. beg. 52 y. ago f. 12 y. Chorea	..	Ab. 200 lesions of Bowen's d. beg. 8 y. after start. As. 8 y.; later more les. trunk and limbs	..
56	Hofmann (1928)	M.	As. f. a long per. Psor.	Ker. extremit. esp. feet, back	Several feet, back, thigh	..
57	Ramel (1929)	M. 68	As. pills more than 30 y. ago	Ker. hands and feet, warty	One: hand, on ker. (after contusion)	..
58	Cole and Driver (1929)	M.	As. (probably) 20 y. f. an eruption	Ker. hands and finger	One: l. p. 20 months	..
59	Konrad (1929)	M. 36	As.-containing mixt. s. 20 y.	Ker. p. and s. s. 10 y.	One: r. s.	Sq. c. carc.
60	Fuhs (1930)	F. 65	S. Fow. f. more than 20 y. Psor.	Ker. hands and feet s. 18 y.	One: r. thenar Sev. ulc. hands and feet on ker.	Sq. c. carc. Metas.
61	Rasch (1930)	..	As. 30 y. Psor.	Ker. soles. Pig.	Two: l. calf	Sq. c. carc.
62	Haagensen (1931) ("case 1") (photo)	M. 55	S. Fow. f. the last 15 y. w. interv. 4–10 dr. *t.i.d.* Psor.	Ker. p. and s. 4 y. after starting As.	Six: fingers of both hands	Sq. c. carc.
63	Haagensen (1931), "case 2"	M. 35	S. Fow. 8 y. Pruritus	Ker. and fiss. p. and s.	One: l. thumb	Sq. c. carc. Metas.
64	Haagensen (1931), "case 3"	M. 46	S. Fow. beg. 16 y. ago periodically. Psor.	Nothing noted	One: l. upp. arm One: l. neck, both s. 4 y.	Bas. c. car
65	Haagensen (1931), "case 4"	M. 31	S. Fow. 14 y. ago, 30 dr. day f. 6 months. Nervousness	Ker. p. and fingers warty, s. 14 y.	One: nr. anus, s. ab. 2 y. Numerous superf. les. trunk, legs, 12 y. aft.; s. ab. 2 y.	Sq. c. car and dermatitis.
66	Stillians (1931)	M. 58	S. Fow. 30 y. prev. f. 2 y. Psor.	*Nil* noted	Sev.: trunk One: forehead One: r. arm.; s. many y.	..
67	Doty, C. A. (1931)	M. 69	S. Fow. large doses 40 y. ago, and 30 pints "psoriasis-medic."	No ker. General pig.	A number of small les. trunk, some ulcerated (prob. superf. ep., see Anderson, 1932) (Bowen's dis. ?)	..
68	Gross, P. (1931)	F. 48	3 y. psor. S. Fow. f. a long time. Skin dis. in childhood	No ker. No pig.	Mult. superf. epith. s. 20–35 (?) y., 3 in face, mult. on trunk	Bas. c. car
69	Milian, G. (1931), "case 1" (see also Milian, 1929)	F. 47	As.	Ker. Pigment. Erythrodermia exfoliativa	Proliferat. of a naevus (melanot. sarcoma ?) 7 y. later: epithelioma perlé (cheek)	Sq. c. car
70	Milian, G. (1931), "case 2"	F.	As.	Ker. not noted. Erythrodermia exfoliativa	Carc. of r. breast	..
71	Fischer, W. (1932)	M. 50	As. long time. Psor.	Ker. p. and s.	Several: r. p. and l. calf	Sq. c. car
72	Andrews, N. P. (1932)	F. 75	S. Fow. 60 y. over unknown per.	Nothing noted	Mult. superf. ep. back, chest, thighs, 40 y.	Bas. c. car
73	Andrews, N. P. (1932) (disc.)	F.	As. Psor.	Nothing noted	Mult. superf. ep. (including hands)	..

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
74	Anderson, N. P. (1932), "case 1" (picture) (see Montgomery, 1935, p. 229)	M. 75	S. Fow. 60 y. ago over unknown period	Ker. p. and s., back of hands, chest. Cutaneous horn, groin. Seborrhoic keratoses	Numerous les. lower part of r. leg and foot, begin. ab. 20 y. ago (?). Mult. superf. ben. ep. back, buttock (Bowen's dis. or arsenic type of ep. ?)	..
75	Anderson, N. P. (1932), "case 3"	M. 70	As. 17 or 18 y. ago as tonic (nerv. breakdown)	Ker. p. and s.; s. 2 y. Seborrhoic keratoses	One lesion of ben. superf. ep. forehead s. 5 or 6 y.	Bas. c. carc.
76	MacCormac, H. (1933*a* and *b*)	F. 51	As. (?) 25 y. ago f. 2 y. Asthma	Ker. p. and s. (punctate). No pig.	Mult. superf. ep. trunk and premalignant warty nodules arms, waist, abdomen. Ca. cervicis uteri	Bas. c. carc.
77	Barber (1933) (discussion to MacCormac, 1933*b*)	M.	As. large doses 20 y. prev. long time. Dermatitis herpetif.	Ker. p. and s. punctate. Melanosis	Mult. superf. ep.	Bas. c. carc.
78	Guggenheim, L. (1933)	F. 55	S. Fow. f. 27 y., 5 drops 2 *t.d.* (25 g. in 27 y.). Epilepsy	Ker. p. and s. Melanosis	Mult. (13) les., polymorph, beg. 15 y. aft. start As.; not on p. and s. Bowen-like	1 Cancroid, 1 bas. c. carc., atyp.
79	Wright and Friedman (1933), "case 2" (picture)	M. 45	Drops, app. As. 15 y. prev. f. 6 weeks. Psor.	No ker. No pig.	Twelve superficial ep. Seven back, first s. 9 y. Three: l. arm One: chest One: l. temple	Mixed bas. and sq. c. carc.
80	Wright and Friedman (1933), "case 4"	F. 40	As. over 20 y. ago f. 2 y. Psor.	No ker. No pig.	Mult. superf. ep. w. pearly border One: forehead Two: back	..
81	Wright and Friedman (1933), "case 5"	F. 60	As. 20 y. prev. f. 1 y. Psor.	No ker. No melanos.	Three superf. ep.: One: leg, s. 13 y. One: neck One: chest	..
82	McNeer (1934), "case 1"	M. 31	S. Fow. 22 y. ago, 30 drops d. f. 6 m.	Ker.	Many les. beg. 5 y. ago nr. anus and other parts	Sq. c. carc.
83	McNeer (1934), "case 2"	M. 50	S. Fow. 20 y. ago, 5 drops 3 *t.d.* f. 3 weeks. Psor.	Ker. hands and extr. trunk	Numerous lesions: hands, upp. extr., trunk, beg. 9 y. ago	Sq. c. carc.
84	McNeer (1934), "case 3"	M. 37	As., beg. 17 y. ago	Ker. p. and s., dors. of hands and feet	One: r. thumb, on wart 8 y. after start. As.	Metas.
85	Ayres, S., Jr. (1934)	M. over 60	S. Fow. Eczema (as student)	Ker. p. and s. (warty) and on trunk beg. 19 y. ago. Telangiect.	One: r. foot on ker. Four: l. heel Mult. ben. superf. ep. w. pearly border, trunk, arms, legs, plus Bowen's dis. (?)	..
86	Wilhelm (disc. Ayres, 1934)	M.	S. Fow. f. sev. y. Epilepsy	Ker. p.	Superf. ep. trunk	..
87	Franseen and Taylor (1934), "case 2"	M. 55	As. f. 3–4 y. and arsphenamine. Dermatitis of scalp	Ker. p., hands, feet, trunk	Mult. epidermoid carc., app. on ker. l. index, l. thumb, r. foot, back, chest, shoulder, heel	Sq. c. carc. Death.
88	Franseen and Taylor (1934), "case 4"	F. 67	S. Fow. 40 y. prev. for constitution	Ker. p., arm, trunk	One: ov. cr. ilei, on ker. s. 7 y. One: neck, papillary. One: r. thigh Susp. carc. oesophagi	Sq. c. carc.

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
89	Franseen and Taylor (1934), "case 5"	M. 63	S. Fow. 10–15 y. prev., 5 drops *t.i.d.* for 2 y. Psor.	Ker. p.	27 lesions, back, trunk, thigh One: l. shoulder	Sq. c. carc.
90	Franseen and Taylor (1934), "case 6"	F. 50	"Dr. Greene's Nervine" 29 y. ago. Nervousness, epilepsy	Ker. whole body (incl. mons pubis)	One: l. middle finger and l. index (papillary) One: neck on ker. One: l. foot (suspic.)	Sq. c. carc. Metas. Death.
91	Franseen and Taylor (1934), "case 7"	M. 51	As. 2 m.	Mult. ker. whole body, hands and feet	One: l. groin	Sq. c, carc.
92	Franseen and Taylor (1934), "case 8"	M. 53	As. 15 y., 6 drops 3 *t.d.* Skin dis.	Ker. p. and s., hands, warty face	One: l. thumb on ker. One: l. ring finger	Mixed bas. sq. carc.
93	Franseen and Taylor (1934), "case 9"	M. 47	S. Fow. quite a period. Epilepsy	Ker. p. and s. shortly after stopp. As.	Carc. pancreat.	..
94	Franseen and Taylor (1934), "case 10" (picture)	M. 37	S. Fow. beg. 14 y. ago, up to 15 drops 3 *t.d.* f. 10 y. w. interv. Psor. (hered.)	Ker. p. and s. beg. 8 y. after starting As.	One: back of hand, beg. 13 y. after starting As.	Sq. c. carc.
95	Franseen and Taylor (1934), "case 16"	M. 34	S. Fow. Asthma	Two ker. temp. reg.	One over the first thoracic vert.	Bas. c. carc.
96	Franseen and Taylor (1934), "case 17"	F. 53	As. 32 y. prev. f. 3 y. off and on. 10 y. prev. f. 2–3 months. Psor.	Ker. trunk, no ker. p. and s.	Beg. age of 51 One: nose One: forehead One: l. temp. reg One: r. upp. arm One: l. upp. arm Two: hip	Ep. aden.-cystic. Bas. c. carc. Metas. Death.
97	Franseen and Taylor (1934), "case 19"	M. 53	S. Fow. in boyhood f. a short t. Acne ?	No ker. p. and s.	One: back, with pearly border Sev.: back One: ulc. above nipple	..
98	Waugh, J., and Scull (1935)	F. 58	S. Fow. 5–7 drops 3 *t.d.* f. more than 3 y. Ataxia ?	Ker. p. and s. punctate. Pig.	Mult. (ab. 12) superfic. ep. on the body, esp. reg. iliaca	Bas. c. carc.
99	Parkhurst, H. J. (disc. to Waugh and Scull, 1935), "case 1"	..	As. from time to time. Psor.	Ker. hands	Superf. epitheliomatosis widespread	..
100	Parkhurst, H. J. (*ib.*), "case 2"	..	As. Psor.	Ker. hands	Superf. ep.	..
101	Robinson, S. (1935)	..	S. Fow. s. 12 y. Psor.	Ker. p. and s., punctate	One: nose	Bas. c. carc.
102	Montgomery, H. (1935), "case 1"	M. 34	S. Fow. beg. 10 y. ago f. 5 y., 1–26 drops 3 *t.d.*, total 1200–1450 c.c. Psor.	Ker. p. and s. s. 7 y. Pig.	One: sole papillom. One: l. foot on ker. Sev.: l. palm One: nr. anus One: scrotum	Sq. c. carc. Metas. Sq. c. carc. Sq. c. carc. Sq. c. carc.
103	Montgomery, H. (*ib.*), "case 2"	M. 42	S. Fow. in early childhood f. 6 y. Eczema	Ker.	One: l. s. fr. ker. 8 y. ago 7 y. later: num. superf. ep. on trunk, and one superf. ep. Later: intraur. ep. Later: bronchial carc.	Sq. c. carc. Sq. c. Bowen Bas. c. carc. Sq. c. carc. Death.

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
04	Montgomery (1935), "case 4"	M. 63	"Ascato" 30 y. prev. Asthma	Ker. p., fingers, 20 y. after start. As. No pigment. Verruc. senil.	Several p. and fingers, some on ker. s. more than 6 y. One: scalp More than 50 superf. ep. trunk "arsenical type" 6–7 y.	Sq. c. carc. One: bas. c. ep. changing in sq.
05	Montgomery (1935), "case 5"	F. 44	S. Fow. 30 y. ago, 3–16 drops 3 *t.d.* f. more than 1 y. Chorea	*Nil* noted	One: r. axilla 15 y. "arsenical type simulating Bowen"	..
06	Montgomery (1935), "case 6"	M. 50	S. Fow. as a child. Chorea	Ker. p. and s. Slight pig.	Num. superf. ep. One: a typical verruc. lesion	..
07	Trow (disc. to Montgomery, 1935)	M. 50	S. Fow. f. 2–3 y. Fits	Ker. p.	One: trunk and back Two: back of fingers app. on ker.	..
08	Rosen (disc. to Montgomery, 1935)	M. 40	Arsphenamine f. ulc. in the mouth	..	Oral mucosa	..
09	Alderson (1935)	M. 50	As. pills, 15 ! daily, each cont. 0·1–0·4 mg., beg. 27 y. ago, f. 7 y. Psor.	Ker. p. and s. (corns). Ker. trunk, nose, ear, eyelid. Pig.	Mult. polymorph. plaques over the body One: abdom., superf., later malign. One: fourth finger later malign. One: neck	..
10	Ayres, S., Jr. (1935)	F. 59	S. Fow. 49 y. ago over a long per. Psor. (?)	Ker. p. and s. (punct.)	Superf. ep. s. 12 y. Two (or more ?): face, forehead, neck, scalp; sim. Bowen	..
11	Bloom (1936)	F.	As. Psor.	Ker. p. and s. No pig.	Superf. ep. back Ep. p. and s. on ker.	.. Sq. c. carc.
12	Fassrainer (1936) (see also Straaten, 1935)	M. 35	S. Fow. 1½ y. Eczema, hands	Ker. hands (warts)	One: hand, from a wart	Bas. c. carc. Metas.
13	Anderson (1937)	M.	"Ascato" 2–3 y. Asthma	Ker. p. and s.	One: l. index 5 y. later: r. index beg. as a wart Mult. ben. superf. ep. scattered over trunk and upp. port. of arms	Sq. c. carc.
14	Ayres, S., Jr. (disc. to Anderson, 1937)	..	As.	Arsenical ker.	One: palm One: axill.	Bas. c. carc. Bas. c. carc. Metas. (sq. c. ep.)
15	Traub (1937)	F. 68	As. 40 y. ago f. 4 y. Acne, psor.	Ker. (?) leg	Mult. polymorph. les. trunk, leg, face beg. 4 y. after medic. of As. One: back	Bas. c. carc. Sq. c. carc.
16	Robinson (1937)	M.	S. Fow. f. many y. Psor.	Ker. p. and s. and body s. 1½ y. Pig.	One: l. sole (ulcus). Pigmented macular scaling areas over the body	..
17	Rauschkolb (Arday) (1938)	M. 49	S. Fow. f. 25 y. off and on. Psor.	Ker. p. and s. Telangiect.	About 50 superf. ep. trunk, upp. extr. beg. 8 y. ago	Bas. c. carc.
18	Barber (1939)	M. 37	As. f. 10 y. Epilepsy	Ker. p. and s.	Numer. superf. ep. trunk	Bas. c. carc.
19	Haldin-Davis (1939), disc. to Barber (1939)	F.	As. f. 20 y. Epilepsy	Ker. p. and s., etc. Pig.	At least one: abdomen	..
20	Voss (1939)	F. 62	S. Fow. As. pills 20 y. ago. Psor.	Ker. trunk but not p. and s. Pig.	18 polymorph. les. trunk, reg. genit. rima ani, thigh, ab. 7 y.	Bas. c. carc.

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
121	Ryan (1939)	M. 52	As. over 12 y. Stomach trouble	Ker. p. and s. and scatt. of tr. and extr. s. 16 y.	Mult. superf. ep. (?) One : r. ear One : chest, trunk (Bowen-like)	..
122	Goeckerman and Wilhelm (1940)	M. 70	S. Fow. f. 10 y. Epilepsy	Ker. p. and s. 14 y. after starting As. 2 y. later trunk (Bowen-like)	Papilloma ureteris et vesicae	..
123	Anderson (disc. to Goeckerman and Wilhelm, 1940)	M. 21	As. 7 y. prev. f. 6 m.	Ker. p. and s.	Mult. superf. ep. trunk One large fungating in groin Carc. of tongue	Sq. c. carc.
124	Applestein (1941)	F. 60	S. Fow. beg. 35 y. ago ; f. 25 y. 5–10 drops 3 *t.d.* Psor.	Nothing noted ab. ker. Pig. Telangiect.	Mult. : epitheliomatosis One : l. shoulder s. 8 y. One : r. temple One : chest, papill., prob. not malign. Forehead, thighs, r. elbow, scalp	Bas. c. carc.
125	Rodgers (1941)	M. 55	S. Fow. many y. ago	Nothing noted ab. ker.	Mult. ben. superf. ep. s. 4 y. L. shoulder Little finger Many : back of hands, scalp, r. knee and legs Back (cauliflower-like)	Sq. c. carc. Bowenoid ? Bas. c. carc. Mixed c. carc.
126	Robinson (disc. to Rodgers, 1941)	..	As. (?)	Arsenical ker. finger, etc.	One : finger	Sq. c. carc.
127	Montgomery and Waisman (1941), "case 1"	M. 49	S. Fow. 26 y. at least once yearly until 9 y. ago. Psor.	Ker. p. and s. s. 14 y.	Sev. : l. p. on ker. (thumb), 15 months ago Numerous trunk and upp. extr. One : r. cheek One : r. fossa, nasal One : r. temple, papillomat. w. pearly border	Sq. c. carc. Sq. c. carc., ars. type; some Bas. c. carc. Bas. c. carc. Bas. c. carc.
128	Montgomery and Waisman (1941), "case 2"	F. 69	S. Fow. 20 y. ago f. 5 weeks and 1 y. ago for 1 week	Num. ker. trunk and extr. (nothing noted of p. and s.). No pig.	Num. lesions trunk and extr. s. 2 y. One verruc. les. on little finger s. 5 y.	Sq. c. carc. (Bowen-like). Sq. c. carc.
129	Montgomery, R. M. (1942)	F. 42	S. Fow. 30 y. ago. Acne	Ker. fingers, soles (punctat.). Leucopl. oris	Multiple : dors. of r. foot, l. knee, l. thigh, dors. of l. hand, wrists s. 10 y.	Bowen ds. (biopsy).
130	Peck (1942)	M. 45	As. drops 3½ y. Psor. hered.	Ker. p.	Two : l. p. on ker.	Sq. c. carc.
131	Ayres, S., Jr. (1943)	M. 56	Asiat. pills 33 y. ago f. 5 y. Psor.	Ker. p. and s. (punctate)	Ab. 30 ben. superf. ep. polym orph., trunk, r. rm. f. 8 y.	Some : sq. c. carc.
132	Anderson (1943)	F. 47	As. inject. 20 y. prev.	Ker. s., no p.	Dry scaly lesions trunk, s 3–4 y. L. shoulder L. hip	Bas. c. carc
133	Ebert and Otsuka (1943)	F. 42	As. as child Anaemia	..	One : r. hip s. 14 y. One : l. chest	Bowen d. c. intraepid. ep.

TABLE I (*continued*).

No.	Author.	Sex. Age.	Drug. Disease.	Keratoses. Pigmentation.	Localization of epitheliomas.	Biopsy. Metastases. Death.
1	Anderson (1943)	M. 50	As.	Ker. p. and s. (punctate)	One: superf. ep. r. shoulder Later: two, scrotum	Bowenoid, Pagetoid.
35	Wilhelm and Goeckerman (1943)	M. 72	As. drops and inject. 10 y. ago	No ker. Pig.	Mult. ben. superf. ep. trunk	Probably bas. c. carc.
36	Laymon (1943)	F. 61	As. drops 17–22 (?) y. prev. f. 5 y. Anaem. pernic.	Ker. p. and s.	At least 30 superf. ep. trunk	..
37	Hall (1944)	F. 61	As. drops (?) small quant. 45 y. ago f. short time (only 7·5c.c.) f. complexion. Later As. for syph. orally and i.v.	No ker. p. and s.	Several superf. ep. s. 11 y. One: scap. reg.	..
38	Barney (1944)	M. 46	Black pills 20 y. ago intermitt. f. 6 months. Psor.	Ker. p. and s. one y. later. Ker. trunk	One: beg. in patch of psor. 10 y. ago Num. superf. polymorph. ep. trunk, extrem. One: below axill. One: scrotum Later: carc. oesophag.	 Bas. c. carc. Sq. c. carc.
39	Dowling (disc. to Glen, 1945)	Old	As. years before. Psor.	Nothing noted ab. ker.	Numerous flat ep.	Sq. c. carc. ? Death, general carc.
40	Russell and Klaber (1945), "case 1"	F. 53	S. Fow. 3 drops 3 *t.d.* beg. 13 y. ago; total 37,000 drops. Epilepsy	No ker. p. and s. Pigment. (no "raindrop"). Mult. seborrh. warts, mollus.	Polymorphic lesions One indurated ulcer chest 12 y. after starting As. Two plaques with pearly edge over sternum	 Sq. c. carc.
41	Russell and Klaber (1945), "case 2"	F. 44	S. Fow. in Br-mixt. f. 30 y.; total 79,000 drops—700 gr. As_2O_3. Epilepsy	Ker. p. No raindrop-pigment. Mult. seborrh. warts and mollus. fibros.	Mult. ep. w. pearly rolled edges trunk, shoulder, thighs Indurated plaque r. f. iliaca One: r. nipple	 Bas. c. carc. (cyst. deg.).
42	Semon, H. C. (disc. to Russell and Klaber, 1945)	M.	S. Fow. large dos. up to 1 dr. 3 *t.d.* Dermatitis herpetiformis	..	Carc. bronch. (skin not affected)	..
43	Semon, H. C. (*ib.*), "case 2"	..	As. for 14 y. Psor.	Ker. feet	One: l. foot on ker.	Sq. c. carc. Death.

Table II.

	Keratoseogen except comb. w. mult. superf. (53 cases).	Mult. superf. and Bowen except comb. w. kerat. (48 cases).	Combination of keratoseogen and mult. superf. (13 cases).	Epith. on patches of psor. (7 cases).	All cases (136 cases).
Fingers, hand, wrist, toes, foot, ankle	45 = 84·9%	4 = 8·3%	13 = 100%	..	63 = 46·3%
Arm, thigh, leg	6 = 11·1%	21 = 43·7%	4 = 30·8%	6 = 85·7%	41 = 30·1%
Scrotum	3 = 5·7%	1 = 2·1%	..	2 = 28·6%	6 = 4·4%
Penis	1 = 1·9%	..	..	..	1 = 0·7%
Vulva	1 = 1·9%	1 = 2·1%	..	..	2 = 1·5%
Pubes, groin, perineum, anal reg.	3 = 5·7%	3 = 6·25%	1 = 7·7%	3 = 42·9%	11 = 8·9%
Neck	5 = 7·5%	4 = 8·3%	..	..	8 = 5·9%
Head	3 = 5·7%	13 = 27·1%	1 = 7·7%	..	19 = 14·0%
Trunk, icl. shoulder, axill., breast, hip, buttock, sacr. reg.	8 = 15·1%	40 = 83·3%	10 = 76·9%	1 = 14·43%	63 = 46·3%
"All over the body"	1 = 1·9%	3 = 6·25%	2 = 15·4%	..	7 = 5·1%
Extracutan.	3 = 5·7%	2 = 4·2%	1 = 7·7%	..	12 = 9·6%

In this Table different localizations in the same region are counted only as one even if, e.g., both hands and both feet are affected.

Table III.

	All cases.	Males.	Females.
20–30 years	10 cases = 8·7%	5 cases = 6·25%	5 cases = 14·3%
31–40 „	28 „ = 24·3%	19 „ = 23·75%	9 „ = 25·5%
41–50 „	35 „ = 30·4%	25 „ = 31·2%	10 „ = 28·6%
51–60 „	24 „ = 20·9%	16 „ = 20·0%	8 „ = 22·9%
61–70 „	17 „ = 14·8%	14 „ = 17·5%	3 „ = 8·6%
71–80 „	1 case = 0·9%	1 case = 1·25%	..
	115 cases	80 cases	35 cases

Table IV.

Hamilton (1921, case 38)	Traces
Schwartz (1926, case 49)	0
Anderson (1932, case 24)	0·00025 mg.%
„ („ „ 25)	0·007 mg.%
Ayres, Jr. (1934, case 85)	0·0027–0·0048 mg.% (in dried substance)
Montgomery (1935, case 97)	0·035 mg.%
„ („ „ 99)	0
Anderson (1937, case 113)	0·037 mg.%
Montgomery and Waisman (1941, case 122)	0
„ „ („ „ 128)	0

Table V.

	As_2O_3 in growth.		As_2O_3 in normal skin.	
	Chem.	Osborne.	Chem.	Osborne.
Case 102, keratoseogen ep. (sq.)	0	+	..	..
„ 103, „ „ (sq.)	0	+	0	0
„ 104, mult. ep. as. type				
(*a*) hand (sq. ep.)	..	++	..	+
(*b*) trunk (sq. ep.)	..	++	..	..
(*c*) back (sq. ep.)	8, 6 mg. %	+	..	..
„ 105, mult. ep. as. type (sq.)	0·18 mg. %	++	..	+
Case of superf. ep. (1935, p. 232) (bas. c.)	0	+	0	+
Ditto (bas. sq.)	0	+	0	+
Ditto (Bowen)	0	0	0	0
Case 106, ben. superf. ep.				
(*a*) bas. c. ep.	0·5 mg. %	..	0·77 mg. %	..
(*b*) Verrucous les. (sq. c.)	..	++	..	+
Case 127, superf. ep. as. type				
(*a*) several (sq. and bas. c.)	0.024–430 mg. %	..	0·008 mg. %	..
(*b*) thenar (sq. ep.)	..	++	..	+
Case 128, num. les. as. type trunk, arms (sq. ep.)	2·2–27·5 mg. %	+	100 mg. % (?)	..

TABLE VI.—*Occupational Arsenical.*

ase.	Author.	Sex. Age.	Employment.	Keratoses. Pigmentation.	Localization of epithelioma.	Biopsy. Metastases. Death.
INERS, SMELTERS :						
1	Anderson (1932), "case 2," fig. 3. (See also Ayres and Anderson, 1934)	M. 51	34 y. previous in smelters in Mexico for 8 y.	Ker. p. and s.	Mult. ben. superf. ep. chest and back s. 7 years	Bas. c. carc. and dys-keratoses.
2	Schaerrer (1934)	..	In silver mining or smelting	..	..	Sq. c. carc. Metas.
3	Migavi (1935)	..	16 y. in smeltery in Japan	Ker. p. and s. 10 y. after starting work	Sq. c. ep. Metas.	..
4	Andrews (disc. to Goeckerman and Wilhelm, 1940)	M.	In a copper mine	Many typical ker. p. and s.	Epitheliomas	..
HEEP-DIP WORKERS :						
5	Eve (1913). (See also O'Donovan, 1924, "case 2")	M. 40	20 y. in sheep-dip fact. Pig. of entire skin	Ker. p. (not s.)	One over l. clav. 2 y.	Sq. c. carc.
6	Porter (1913). (See also O'Donovan, 1924, "case 3")	M. 35	20 y. in sheep-dip fact.	Pig. all over the body	One : l. shoulder beg. as a wart 8 months prev.	Sq. c. carc. Death.
7	Legge (1923), "case 1"	M. 53	38 y. in sheep-dip fact.	Warty ker.	One abdomen	Carc. not sure.
8	Legge (1923), "case 2"	M. 53	33 y. in sheep-dip fact.	Ker. p.	Mult. growths armpit, groin, thigh, scrotum, sacrum, arms, feet	..
9	Legge (1923), "case 3"	M. 39	22 y. in sheep-dip fact.	Ker. p.	Warty conditions back of hands, r. groin, l. buttock	Carc. not sure.
10	Legge (1924, 1934), "case 4" O'Donovan (1924*a*, 1928), "case 1" (picture)	M. 44	26 y. in sheep-dip fact.	Ker. warts	One : l. temple on a wart. Four growths prev., l. clavicle, abdomen, l. flank, thigh	Sq. c. carc.
11	Bridge (1926)	..	..	..	..	Metas. death.
12	Bridge (1926)	..	..	..	..	Metas. death.
13	Bridge (1930)	M. 61	46 y. in sheep-dip fact.	..	Subsequent primary growths :— One : r. shoulder (36 y. after beg. employm.) One : neck (5 y. later) One : l. upper arm (5 y. later) One : penis	..
14	Legge (1934), "case 3"	M. 49	22 y. in sheep-dip fact.	Ker. p. Pig.	Warts back of hands, r. groin, l. buttock	..
15	Merewether (1943)	M. 57	43 y. in sheep-dip fact.	..	..	..
16–18	Merewether (1943)	..	In sheep-dip fact.	..	..	..
NSECTICIDES :						
19	Legge (1925) Bridge (1928)	M. 48	19 y. in emerald green fact. and 10 y. further exposure to As.	Pig.	One : l. axilla. 2 y. later, one below l. knee	Metas. death.

TABLE VI.—*Occupational Arsenical (continued).*

Case.	Author.	Sex. Age.	Employment.	Keratoses. Pigmentation.	Localization of epithelioma.	Biopsy. Metastases. Death.
20	Hopkins and Van Studdiford (1934) (picture)	M. 5 Negro	Living in cotton wool farm; fields sprayed with insecticides	Ker. p. Pig. hands.	Mult. ep. face s. 4 y., exulcerated	..
21	Franseen and Taylor (1934) "case 1"	M. 59	30 or more y. employed w. As-prep. f. fruit trees	Ker. p. a. *s.*	Mult. scaly les. ov. the body s. 40 y. 4 bas. c. ep. ear, upp. lip, arm, leg	Resid.
22	Franseen and Taylor (1934) "case 3"	M. 51	27 y. ago empl. w. As-prep. for fruit trees	Ker. p. warty	One carc. neck. Mult. carc. trunk	..
23	v. Pein (1938*a*, *b*)	M. 40	Vintager, 4 y. use of "Nospran"	Ker. p. a. s.	Mult. ulc. carc. skin	..
24	Bridge (1939)		Empl. in factory of As-containing insecticides	Melanosis, Perfor. of nasal sept.	Warty growths ov. the body. Primary carc. of r. lung	..

ABBREVIATIONS.

As., arsenic; bas., basal; beg., beginning; betw., between; carc., carcinoma; c., cell(ed); ep., epithelioma; interv., interval; ker., keratosis; l., left; les., lesion(s); m., month; metas., metastases; mult., multiple; nr., near; num., numerous; p., palms; per., period; pig., pigment(ed); psor., psoriasis; r., right; rec., recurrence; s., soles; S. Don., Sol. Donovan; S. Fow., Sol. Fowleri; sev., several; sq., squamous; superf., superficial; ulc., ulcer(ated); y., years.

cancer in Table I is the occurrence of malignancy in other members of the family mentioned (cases 4 and 104, in neither of them affecting the skin). Such occasional coincidence of course cannot prove anything. On the other hand a systematic investigation of the hereditary conditions in arsenical cancer has never been carried out.

The possible role of *embryonic germs* in the sense of the Cohnheim theory of cancer was the starting-point of the above-mentioned experiments of Carrel (1925) and Askanazy (1926).

Askanazy, stressing the necessity to assume the *co-operation of several factors* for the genesis of arsenical cancer, ascribed to arsenic the role of an irritant of oligodynamic efficiency, acting alongside a general constitutional predisposition ("allgemeine Koerperbereitschaft") and a local predisposition (e.g. presence of embryonic tissue). Fischer-Wasels (1928, 1929) and Buengeler (1930), on the other hand, suggested that arsenical poisoning after a latent sensitizing period causes a general disposition for cancer ("allgemeine Geschwulstdisposition"), marked by certain metabolic changes (see p. 228), preparing the ground for the development of malignancy out of a local anlage ("locale Geschwulstanlage").

Given such a general disposition, or provided even pre-cancerous lesions (keratosis) have developed, then the subsequent development of an epithelioma at that site may be facilitated by the action of *non-specific external irritation*,

such as by rubbing, scratching, or other slight traumatism (Wile, 1912; de Silva, 1918).

Eggers (1931), summarizing the facts, concludes that arsenic is an agent that seems to cause a decided increase in the predisposition to cancer, so that an added element of irritation, which in ordinary conditions would be inadequate, comes into operation.

This work was carried out with the aid of a grant from the Medical Research Council.

REFERENCES.

ABRAMOWITZ, E. W., MATTICE, M. R., AND BOTTVINCK.—(1944) *Arch. Derm. Syph., Chicago*, **47**, 175.

AITKEN, R.—(1944) *Edinb. med. J.*, **51**, 339.

ALDERSON, H. E.—(1935) *Arch. Derm. Syph., Chicago*, **32**, 313.

ALEXANDER, A.—(1921) *Arch. Derm. Syph.*, Wien, **129**, 5.

ALIFERIS.—(1924) *Ibid.*, **147**, 349.

ALTHAUSEN AND GUNTHER.—(1929) *J. Amer. med. Ass.*, **92**, 2002.

ALVAREZ, C.—(1928) *Rev. méd. Rosario*, **18**, 171.

Idem AND RUIZ, E. R.—(1927) *Ibid.*, **17**, 115.

ANDERSON, N. P.—(1932) *Arch. Derm. Syph., Chicago*, **26**, 1052.—(1937) *Ibid.*, **35**, 323.—(1940) *Ibid.*, **42**, 641.—(1943*a*) *Ibid.*, **47**, 136.—(1943*b*) *Ibid.*, **48**, 319.

Idem AND BURPEAU, C.—(1945) *Ibid.*, **52**, 46.

ANDREWS, N. P.—(1932) *Ibid.*, **25**, 572.

ANTONOFF, A. M.—(1928) 'Problems of Oncology,' Kharkoff, VI, 292. Ref. in *Cancer Rev.* (1930), 108.

APPLESTEIN, R.—(1941) *Arch. Derm. Syph., Chicago*, **43**, 599.

ARDAY, E. J.—(1938) *Ibid.*, **37**, 331.

ARGUELLO, R. A., CENGET, D., AND TELLO, E. E.—(1938) *Rev. Derm. Argent.*, **22**, 461; ref. in *Brit. J. Derm.* (1939), **51**, 548.

Idem, FERRARIS, L. V., AND TELLO, E. E.—(1938) *Rev. Derm. Argent.*, **26**, 313.

Idem AND TELLO, E. E.—(1943) *Ibid.*, **27**, 152.

Iidem AND MARCOLA, B.—(1942) *Ibid.*, **26**, 1155.

ARNING.—(1922) *Arch. Derm. Syph., Wien*, **138**, 458.

ARNSTEIN, A.—(1913*a*) *Verh. deutsch. path. Ges.*, **16**, 332.—(1913*b*) *Oesterr Sanitaetswesen*, 38, *Wien Arb. soz. Med.*, Beihefte **5**, 64.

ASCHOFF, L.—(1887–1889) in 'Klin. Jahrbuch,' 1902.

ASKANAZY, M.—(1926) *Verh. dtsch. path. Ges.*, **21**, 182.

AYRES, S., Jr.—(1934) *Arch. Derm. Syph., Chicago*, **29**, 613.—(1935) *Ibid.*, **32**, 322.—(1937) *Ibid.*, **35**, 323 (discussion).—(1943) *Ibid.*, **47**, 135, 136.

Idem AND ANDERSON, N. P.—(1934) *Ibid.*, **30**, 33.—(1938) *J. Amer. med. Assoc.*, **110**, 886.

BALTHAZARD.—Arsenic in 'Occupation and Health Encyclop.,' Geneva, 1930, vol. i, p. 159.

BANGA, I., SCHNEIDER, L., AND SZENT-GYEORGYI, A.—(1931) *Biochem. Z.*, **240**, 462.

BARBER, H. W.—(1925) *Brit. J. Derm.*, **37**, 37.—(1928) *Ibid.*, **38**, 294.—(1933) *Ibid.*, **45**, 475 (discussion).—(1939) *Ibid.*, **58**, 85.

BARNEY.—(1944) *Arch. Derm. Syph., Chicago*, **50**, 339.

BARRY, G., BUNBURY, E., AND KENNAWAY, E. L.—(1928) *Biochem. J.*, **22**, 1109.

BAYET.—(1923*a*) *Le Cancer*, **1**, 5.—(1923*b*) *Congr. du Cancer, Strasbourg*, **2**, 15.—(1930) 'Occupation and Health Encyclop.', vol. ii, p. 1140.

Idem AND SLOSSE.—(1919*a*) *Bull. Acad. Méd. Belg.*, **29**, 607.—(1919*b*) *C.R. Acad. Sci.*, **168**, 704.—(1919*c*) *C.R. Soc. Biol.*, **82**, 1144.

Begg, A. M., and Cramer, W.—(1929) *Lancet*, ii, 217, 697.
Berenblum.—(1932) *Cancer Rev.*, **7**, 338.
Bierich, R.—(1922) *Z. Krebsforsch.*, **18**, 226, *cit.* Lipschuetz, 1924.
Billeter, O., and Marfurt, *E.*—(1923) *Helv. chim. Acta*, **6**, 780.
Binz, C., and Schulz, H.—(1879) *Arch. exp. Path. Pharmak.*, **11**, 200.
Bland-Sutton, J.—(1916) *Brit. med. J.*, ii, 788.
Bloom, D.—(1936) *Arch. Derm. Syph., Chicago*, **33**, 937.
Bosco, J. J.—(1925) *Semana méd.*, **32**, ii, 951.
Bosellini.—(1928) *Brit. J. Derm.*, **38**, 48.
Bowen, J. T.—(1912) *J. cutan. Dis.*, **30**, 241.
Bridge, J. C.—(1926) *Ann. Rep. Chief Ins. Fact. and Worksh.*—(1928) *Ibid.*—(1930) *Ibid.*—(1939) *Ibid.*
Brooke, H. G., and Roberts, L.—(1901) *Brit. J. Derm.*, **13**, 121.
Bruenauer.—(1921) *Arch. Derm. Syph., Wien*, **129**, 186.
Buengeler, W.—(1930) *Frankf. Z. Path.*, **39**, 314.
Buergener, J.—(1939) *Dermatolog.*, **80**, 86.
Butlin, H.—*Brit. med. J.*, i, 1341 ; ii, 1, 66.
Califano, L.—(1931) *Riv. Patol. sper.*, **6**, 113.
Carlson, C. E.—(1910) *Z. physiol. Chem.*, **68**, 243.
Carrel, A.—(1925) *C.R. Soc. Biol.*, **93**, 1083.
Cartaz—(1878) *Bull. Soc. anat., Paris*, **9**, 587.
Cholewa, J.—(1934/35) *Z. Krebsforsch.*, **41**, 497.
Ciechanowski, Morozowa, F., and Wilhelmi, M.—(1925) *Polska Gaz. lekarska*, 3, **25**, 305 ; ref. *Zbl. allg. Path. path. Anat.*, **36**, 294.
Cole and Driver.—(1929) *Arch. Derm. Syph., Chicago*, **20**, 143.
Collier, W. A., and Hartnack.—(1929) *Z. Krebsforsch.*, Orig., **30**, 131.
Cook, J. W., Haslewood, G. A. D., Hewett, C. L., Hieger, I., Kennaway, E. L., and Mayneord, W. V.—(1937) *Amer. J. Cancer*, **42**, 295.
Crocker, H. R., and Pernet.—(1901) *Brit. med. J.*, ii, 864.
Currie, A. N.—(1947) *Brit. med. Bull.*, **4**, 402.
Daels, F.—(1923) ' Congr. du Cancer, Strasbourg.'
Darier, J.—(1902) *Ann. Derm. Syph.*, 4th ser., t. iii, 1121, *cit.* Pye-Smith, 1913*b*.—(1914/15) *Ibid.*, **5**, 449.—(1920) *Ibid.*, **1**, 49, *cit.* Anderson, 1932.
Deelman, H. T.—(1928) *Ann. Méd.*, **24**, 360.
Dennie, C. C., and McBride, W. C.—(1924) *J. Amer. med. Ass.*, **83**, 2082.
de Silva.—(1918) *Med. Presse*, n.s. **105**, 243.
Doerle, M., and Ziegler, K.—(1930) *Z. klin. Med.*, **112**, 237.
Doty, C. A.—(1931) *Arch. Derm. Syph., Chicago*, **23**, 585.
Dowling, G. B.—(1945) *Proc. Roy. Soc. Med.*, **38**, No. 3, Sect. Derm., p. 127.
Dresel, K.—(1926) *Biochem. Z.*, **178**, 70.—(1928) *Ibid.*, **192**, 351.
Dubreuilh, W.—(1910) *Ann. Derm. Syph.*, 5th ser., t. 1.
Dustin, A. P.—(1930) *C.R. Soc. Biol.*, **103**, 953.
Idem and Gregoire, C.—(1933) *Ibid.*, **114**, 195.
Idem and Piton.—(1925) *Ibid.*, **93**, 1535.—(1929) *Bull. Acad. Méd. Belg.*, 5e sér., **9**, 26.
Ebert, M. H.—(1929) *Arch. Derm. Syph., Wien*, **158**, 365.
Idem and Otsuka.—(1943) *Arch. Derm. Syph., Chicago*, **48**, 413.
Editorial—(1942) *J. Amer. med. Ass.*, **120**, 124.
Eggers, H. E.—(1931) *Arch. Path.*, **12**, 983—(1932) *Ibid.*, **13**, 134.
Erdmann, H.—(1904) *Z. anorg. Chem.*, **32**, 453.
Eve, F. R.—*Cit.* Pye-Smith, 1913*b*.
Fassrainer, S.—(1936) *Zbl. Chir.*, **631**, 23.
Felloni, G.—(1930) *Pathologica*, **22**, 256.
Fernandez, A. A.—(1925) *Semana méd.*, **32**, ii, 485.
Finner, L. L., and Calvery, H. C.—(1939) *Arch. Path.*, **27**, 433.

FISCHER, A.—(1926) *C.R. Soc. Biol.*, **94**, 1217.
FISCHER, W.—(1932) *cit. Dermatol.* (1939), **80**, 86.
FISCHER-WASELS, B.—(1928) *Münch. med. Wschr.*, p. 73.—(1929) *Virch. Arch. path. Anat. Physiol.*, **275**, 723.
FORDYCE, J. A., AND MCKEE.—(1914) *J. cutan. Dis.*, **32**, 446.
FRANSEEN, C. C., AND TAYLOR.—(1934) *Amer. J. Cancer*, **22**, 287.
FRASER, J. F.—(1927) *Arch. Derm. Syph., Chicago*, **16**, 518.—(1928) *Ibid.*, **18**, 809.—(1929*a*) Discussion to Wise, F., *ibid.*, **19**, 1.—(1929*b*) *Ibid.*, **19**, 306.
FROHN, W.—(1938) *Münch. med. Wschr.*, **85**, ii, 1630.
FUHS.—(1930) *Derm. Wschr.*, **90**, 457, *cit. Cancer Rev.*, 1932, **7**, 471.—(1932) *Ibid.*, **94**, 693.
FUNK, C.—(1915) *J. exp. Med.*, **21**, 571, 574.
GARCIA, P. P., AND RUIZ, F. R.—(1928) *Semana Méd.*, **35**, 515, *cit. Cancer Rev.*, 1930, **5**, 7.
GAUVIN.—(1927) *Arch. Derm. Syph., Chicago*, **15**, 350, *cit.* Franseen and Taylor, 1934.
GEYER, L.—(1898) *Arch. Derm. Syph. Wien*, **43**, 221.—(1940) *Klin. Wschr.*, **19**, 750.
GIES, TH.—(1877) *Arch. exp. Path. Pharm.*, **8**, 175.
GLEN, E. M.—(1945) *Proc. Roy. Soc. Med.*, **38**, No. 3, Sect. Derm., p. 127.
GOECKERMAN, W. H., AND WILHELM.—(1940) *Arch. Derm. Syph., Chicago*, **42**, 641.
GRAY, A. M. E.—(1912) *Brit. J. Derm.*, **24**, 325.
GROSS, P.—(1931) *Arch. Derm. Syph., Chicago*, **23**, 1132.
GUGGENHEIM, L.—(1933) *Arch. Derm. Syph., Wien*, **16**, 26.
GUTHMANN, H.—(1938) *Archiv. Gynaek.*, **166**, 526.
GUTZEIT.—(1879) *Pharm. Z.*, **24**, 263.
HAAGEN, E.—(1928) *Dtsch. med. Wschr.*, **54**, i, 92.
HAAGENSEN, C. D.—(1931) *Amer. J. Cancer*, **15**, i, 656–659.
HAERTING, F. H., AND HESSE, W.—(1879) *Vjschr. Med. gerichK.*, **30**, 296 ; **31**, 102, 313.
HALL, A. F.—(1944) *Arch. Derm. Syph., Chicago*, **50**, 148.
HAMILTON, A.—(1925) ' Industrial Poisons in the United States,' New York, p. 206.
HAMILTON, G. R.—(1921) *Brit. J. Derm.*, **33**, 15, 31.
HARBITZ, H. F.—(1927) *Lancet*, **212**, 70.
HARTZELL, M. B.—(1899) *The New Sydenham Soc.*, **170**, 259.
HARTZELL AND STELWAGON.—(1899) *Trans. Amer. derm. Ass.*, p. 11.—*Amer. J. med. Sci.*, **118**, 265.
HAXTHAUSEN, H.—(1923) *Hospitalstidende*, 44.
HEFFTER, A., AND KEESER, E.—(1927) ' Handb. exp. Pharm.', vol. iii, 1 Haelfte, p. 463.—(1937) *Erg-Bd.*, 1937, **3**, 162.
HERMANN, L.—(1879) ' Lehrbuch. d. exp. Toxikologie,' Berlin.
HEUBNER, W.—(1907) *Arch. exp. Path. Pharmak.*, **56**, 370.
HERXHEIMER—(1939) *cit. Dermatol.*, (1939), **80**, 86.
HOFMANN, E.—(1928) *cit. Dermatol.* (1939), **80**, 86.
HOPKINS, R. AND VAN STUDDIFORD, M. T., *cit. Dermatol.* (1939), **80**, 86.
HUECK, W.—(1939/40) *Z. Krebsforsch.*, **49**, 312.
HUEPER, W. C.—(1942*a*) *Cancer Res.*, **2**, 551.—(1942*b*) ' Occupational Tumours,' Baltimore (Thomas).—(1943) *Lancet*, i, 538.
Idem AND ITAMI, S.—(1933) *Amer. J. Cancer*, **17**, 106.
HUTCHINSON, J.—(1887) *Brit. med. J.*, ii, 1280.—(1888) *Trans. path. Soc. London*, **39**, 352.—(1894) *Arch. Surg. Lond.*, 9, **63**, 223.—(1895) ' A Smaller Atlas of Illustr. of Clin. Surg.,' London.—(1898) *Arch. Surg. Lond.*, **9**, 63, 223.—(1902) *Policlinic*, **6**, 336.—(1903) ' Atlas Clin. Med. Surg. and Path.', VI, p. 71.
HYDE, J. N.—(1899) *Trans. Amer. derm. Ass.*, p. 18.
JUSTUS, J.—(1905) *Derm. Z.*, **12**, 277.
KATHE, J.—(1937) ' Oberschl. Ges. vaterl. Kult. Breslau, Mednaturw. R.,' No. 3.
KAUFFMANN, FR.—(1929) *Z. Krebsforsch.*, **30**, 136; ref. in *Cancer Rev.*, **5**, 180.

KENNAWAY, E. L.—(1925) *J. industr. Hyg.*, **7**, 69.—(1942) *Lancet*, ii, 769.—(1943) *Ibid.*, i, 599.
KETRON.—(1927) *Arch. Derm. Syph., Chicago*, **16**, 642, 643.
KOERBLER, J.—(1939) *Liječn Vijesn.*, **61**, 590.
KONRAD.—(1929) *Derm. Wschr.*, **88**, 690.
KROTKINA, N.—(1934) *Vop. Onkol.*, **6**, 119; ref. in *Z. Krebsforsch.*, 1935, **41**, 251.
LABES, R.—(1929) *Arch. exp. Path. Pharmak.*, **141**, 148.
LACLAU, N. C., AND PILLADO-MATHEU, C.—(1930) *C.R. Soc. Biol.*, **103**, 1285.
LANE, C.—(1939) *Arch. Derm. Syph., Chicago*, **40**, 105.
LANE, W. A.—(1894*a*) *Trans. Clin. Soc.*, **27**, 102.—(1894*b*) *Brit. med. J.*, i, 354.
LANGE, K.—(1935) *Z. Krebsforsch.*, **42**, 306.
LA ROSSA.—(1925) *Rass. int. Clin. Terap.*, **6**, 28.
LAYMON, C. W.—(1943) *Arch. Derm. Syph., Chicago*, **48**, 681.
LEGGE, T. M.—(1902) 'Ann. Rep. Chief Insp. Fact. and Worksh.'—(1923) *Ibid.*, (1924) *Ibid.*—(1925) *Ibid.*—(1934) 'Industrial Maladies,' Oxford, p. 84.
LEITCH, A.—(1923) *Brit. med. J.*, ii, 1.
Idem, AND KENNAWAY, E. L.—(1922) *Ibid.*, ii, 1107.
LEVIN, O. L.—(1926) *Arch. Derm. Syph., Chicago*, **13**, 569.
LIPSCHUETZ.—(1924) *Arch. Derm. Syph., Wien*, **147**, 520.
LITTLE, GRAHAM.—(1923) *Brit. J. Derm.*, **35**, 435.
LOEWENBERG, M.—(1912) *Verh. Ges. dtsch. Naturf. Ärzt.*, **84**, p. 72, Hlfte, p. 312 see also Ullmann, 1922.
LOEWY, J.—(1929) *Med. Klinik*, **25**, 141.
LORENZ, E.—(1944) *J. Nat. Cancer Inst.*, **5**, 1.
LUDEWIG, P., AND LORENSER, E.—(1924*a*) *Z. Phys.*, **22**, 178.—(1924*b*) *Strahlentherapie*, **17**, 428.
MACCORMAC.—(1933*a*) *Proc. Roy. Soc. Med.*, **26**, 1553.—(1933*b*) *Brit. J. Derm.*, **45**, 475.
MCCOY, J. N.—(1920) *Arch. Derm. Syph., Chicago*, **6**, 175.
MCJUNKIN, F. A., AND CIRKIT, M. F.—(1929) *Proc. Soc. exp. Biol.*, **27**, 179.
MACLEOD, J. M. H.—(1913) *Proc. Roy. Soc. Med.*, vi, 1, Derm. Sect., p. 109.
MCNEER, G.—(1934) *Ann. Surg.*, **99**, 348.
MAISIN, J.—(1934) *Brux. méd.*, **14**, 544, *cit.* Hueper, 1942.
Idem AND DUPUIS.—(1929) *Rev. belge Sci. méd.*, i, 409.
MANOUVRIER.—(1876) *cit.* Bayet and Slosse (1919*a*).
MATRAS, A.—(1940) *Med. Klin.*, **36**, 1274, *cit. Bull. Hyg., Lond.*, **17**, 383.
MAYER.—(1933) *Handb. d. Haut- und Geschl-krankh.*, **4**, pt. 2, 72, *cit.* Goeckerman and Wilhelm, 1940.
MEREWETHER, E. R.—(1943) 'Ann. Rep. Chief Insp. Fact. and Worksh.,' p. 45.
MIGAYI, S.—(1935) *Zbl. Chir.*, **62**, ii, 2063.
MILCH, H.—(1932) *Amer. J. Cancer*, **16**, 89.
MILIAN, G.—(1929) *Rev. franç. Derm. Vénéréol.*, p. 507.—(1931) *Ibid.*, **7**, 580.
MOELLER, E.—(1923) *Z. Krebsforsch.*, **19**, 393.
MONTEMARTINI, G.—(1928) *Tumori*, **14**, 1; ref. in *Cancer Rev.*, 1929, **4**, 269.
MONTGOMERY, H.—(1929) *Arch. Derm. Syph., Chicago*, **20**, 339.—(1935) *Ibid.*, **32**, 229.
Idem AND WAISMAN.—(1941) *J. Invest. Derm.*, **4**, 365.
MONTGOMERY, R. M.—(1942) *Arch. Derm. Syph., Chicago*, **45**, 407.
MYERS, C. N., AND CORNWALL, L. H.—(1925) *Amer. J. Syph.*, **9**, 647.
NANDER.—(1923) *Hospitalstidende*, **66**, 7.
NIEBERLE, K. (1939)—*Z. Krebsforsch.*, Orig., **49**, 137.
NUTT, W. H., BEATTIE, J. M., AND PYE-SMITH, R. J., *see* Pye-Smith (1913).
O'DONOVAN, W. J.—(1924) *Brit. J. Derm.*, **36**, 477.—(1928) *J. State Med.*, **36**, 700.
O'LEARY, P. A.—(1935) *Arch. Derm. Syph., Chicago*, **31**, 413.

OLIVER.—(1923) *Ibid.*, **8**, 267.
Idem AND FINNERUD.—(1928) *Ibid.*, **17**, 892, *cit. Cancer Rev.*, 1929, **4**, 21.
ONAKA, O.—(1911) *Z. physiol. Chem.*, **70**, 433.
OPPENHEIM, M.—(1931) *Dermatol. Wschr.*, **92**, 741.
OPPENHEIM AND FANTL.—(1934) *Arch. Derm. Syph., Wien*, **170**, 488, *cit.* Montgomery and Waisman, 1941, discussion.
ORMSBY AND MITCHELL.—(1925) *Arch. Derm. Syph., Chicago*, **12**, 144, 426.
OSBORNE.—(1925) *Ibid.*, **12**, 773.—(1928) *Ibid.*, **18**, 37.—(1932) *Ibid.*, **25**, 419.
OSTERBERG, A. E.—(1928) *J. biol. Chem.*, **76**, 19.
Idem AND GREEN, W.—(1944) *Ibid.*, **155**, 513.
OWEN.—(1921) *J. Amer. med. Ass.*, **76**, 1329.
PACK, G. T., AND LEFEVRE, R. G.—(1930) *J. Cancer Res.*, **14**, 167.
PARIS, J. AYRTON.—' Pharmacologia,' 3rd ed., 1820, London, *cit.* Kennaway, 1942.
PECK, S. M.—(1942) *Arch. Derm. Syph., Chicago*, **45**, 1016.
v. PEIN, H.—(1938*a*) *Dtsch. med. Wschr.*, **64**, 565.—(1938*b*) *Münch. med. Wschr.*, 1017.
Idem AND BAURHENN.—(1943) *Klin. Wschr.*, **22**, 388.
PELLER, S.—(1939) *Hum. Biol.*, **11**, 130.
PENTIMILLI, F.—(1926) *Lo Esperimentale*, **80**, 624 ; ref. in *Cancer Rev.*, 1928, **5**, 163.
PETROFF, N., AND KROTKINA, N.—(1928) *Bull. Ass. franç. Cancer*, **41**, 251.
PFAHLER.—(1927) *Arch. Derm. Syph., Chicago*, **15**, 213.
PIRCHAN AND ŠIKL, H.—(1932) *Amer. J. Cancer*, **16**, 681.
PITON, R.—(1929) *Arch. int. Méd. exp.*, **5**, 355.
PORTER, CH. R.—*Cit.* Pye-Smith, 1913*b*.
POSNER.—(1904) *Z. Krebsforsch.*, **1**, 4.—(1905) *Dtsch. Klin.*, **10**, 455.—(1924) *Z. Urol.*, **18**, 418.
POZZI.—(1874) *Bull. Soc. anat. Paris, cit.* Hartzell, 1899.
PRELL, H.—(1936/37) *Arch. Gewerbepath. Gewerbehyg.*, **7**, 656.
PUCCINELLI, E.—(1930*a*) *Pathologica*, **22**, 63.—(1930*b*) *Ibid.*, **22**, 349.
PUTMAN, F. L.—(1945) *Arch. Derm. Syph., Chicago*, **51**, 225.
PYE-SMITH, R. J.—(1913*a*) *Proc. Roy. Soc. Med., Clin. Sect.*, **6**, I, 229.—(1913*b*) *Lancet*, ii, 210, 282.
RAMEL.—(1929) *Schweiz. med. Wschr.*, **10**, 1230, *cit. Cancer Rev.*, **5**, 404.
RAPOSO, S.—(1928) *C.R. Soc. Biol.*, **98**, 86.—(1929) *A. ch. Port. Sci. Biol.*, **2**, 146, 186.
RASCH.—(1930) *Internat. Congr. Derm. Syph., Copenhague*, cit. *Dermatologica*, 1930, **80**, 86.
RAUSCHKOLB (ARDAY, E. J.).—(1938) *Arch. Derm. Syph., Chicago*, **37**, 331.
RAVAUT, P.—(1920) *Pr. méd.*, **28**, 73, *cit.* Franseen and Taylor, 1934.
REMENOVSKI, F.—(1921) *Arch. Derm. Syph., Wien*, **131**, 465.
RINGER, S., AND MURREL, W.—(1878/79) *J. Physiol.*, **1**, 213.
ROBINSON, S.—(1935) *Arch. Derm. Syph., Chicago*, **31**, 260.—(1937) *Ibid.*, **36**, 870, discussion to Traub.—(1941) *Ibid.*, **44**, 78, discussion to Rodgers.
ROCMANS, M.—(1930) *C.R. Soc. Biol.*, **103**, 42.
RODGERS, J. D.—(1941) *Arch. Derm. Syph., Chicago*, **44**, 78.
ROFFO, A. H., AND CORREA, L. M.—(1926) *Bol. Inst. Med. Esp.*, **3**, 367.
Idem AND ROSNER, S.—(1935) *Rev. Ass. méd. Argent.*, **49**, 1931.—(1936) *Semana méd.*, **1**, 861.
ROMBERG.—' Klin. Wahrnehmungen u. Beob.,' 1851.
ROSEN.—(1935) *Arch. Derm. Syph., Chicago*, **32**, 218 ; discussion to Montgomery.
ROSENTHAL, S. M., AND VOEGTLIN.—(1930) *J. Pharmacol.*, **39**, 246.
ROST, E.—(1931) ' Handb. d. Lebensm-chemie,' Berlin, **1**, 1073, *cit.* Wuehrer, 1937.
ROSTOSKI, O., SAUPE AND SCHMORL.—(1926) *Z. Krebsforsch.*, **23**, 360.—(1927) *Ibid.*, **25**, 249.
Idem AND SAUPE, E.—(1931) *Arch. Gewerbepath. Gewerbehyg.*, **1**, 731.

RUSSELL, B. F., AND KLABER, R.—(1945) *Proc. Roy. Soc. Med.*, **38**, No. 3, Sect. Derm., p. 128.
RYAN, M. L.—(1939) *Arch. Derm. Syph., Chicago*, **40**, 104.
SALKOWSKI, E.—(1908) *Z. physiol. Chem.*, **56**, 95.
SAUPE, E.—(1930) *Arch. Gewerbepath. Gewerbehyg.*, **1**, 582.
SCHABAD, L. M.—(1935) *Z. Krebsforsch.*, **42**, 295.
SCHAERRER, W. C.—(1934) *University Western Ontario Med. J.*, **5**, 27.
SCHAMBERG, J. F.—(1907*a*) *J. cutan. Dis.*, **25**, 26.—(1907*b*) *Ibid.*, **27**, 130, *cit.* Pye-Smith, 1913*b*.
SCHILLER, W.—(1926) *Z. Krebsforsch.*, **23**, 9.
SCHINZ, H. R.—(1942) *Schweiz. med. Wschr.*, **72**, 1070.
SCHMIEDEBERG, O.—(1906) ' Grundviss der Pharmakologie,' Leipzig (Vogel) p. 476.
SCHREINER, B. T., AND WEHR, W. H.—(1934) *Amer. J. Cancer*, **20**, 418.
SCHWARTZ.—(1926) *Arch. Derm. Syph., Chicago*, **13**, 705.
SCHWARZ, L.—(1932) *Derm. Wschr.*, **94**, 577.
Idem AND DECKERT, W.—(1943) *Arch. Hyg. Berl.*, **129**, 276 ; ref. *Bull. Hyg. Lond.*, (1943), **18**, 927.
SEMINARIO, C., AND ALVARADO, G. E. R.—(1931) *Semana méd.*, **36**, i, 1665 ; ref. in *Cancer Rev.* (1931) 475.
SEMON, H. C.—(1922) *Brit. med. J.*, 975.—(1945) *Proc. Roy. Soc. Med.*, **38**, No. 3, Sect. Derm., p. 128, discussion.
ŠIKL, H.—(1930) *Z. Krebsforsch.*, **32**, 609.
SIMONS, R.—(1937) ' Arsenicodermie,' Leiden, 1937.
SPENCER.—Discussion to Pye-Smith (1913*a*).
STELWAGON, H. W.—(1910) ' Treatise on Diseases of the Skin,' 6th ed., London.
STEWART.—(1934) *J. Urol.*, **30**, 170 ; *cit.* Hueper, 1942.
STICKER, A.—(1911) *Zbl. Bakt.*, **59**, 464.
STILLIANS, A. W.—(1919) *J. cutan. Dis.*, **37**, 269.—(1930) *Arch. Derm. Syph., Chicago*, **22**, 1085.—(1931) *Ibid.*, **23**, 377 ; *cit.* Anderson, 1932.
STOKES.—*Cit.* Peck (1942).
STRAATEN.—(1935) *Zbl. Chir.*, No. 37.
SUTTON.—(1939) ' Diseases of the Skin,' London.
TANNENHOLZ, N., AND MUIR, K. B.—(1933) *Arch. Path.*, **15**, 789.
THRONE, B., AND MYERS, C. N.—(1935) *Arch. Derm. Syph., Chicago*, **32**, 181.
TILLAUX.—(1877) *Bull. mém. soc. Chir. Paris*, **3**, 351.
TIMBERLAKE, H. P.—(1929) *U.S. Veterans Bur. Med. Bull.*, **5**, 678 ; *cit.* Franseen and Taylor, 1934.
TOEROEK.—(1891) *Ann. Derm. Syph., Paris*, 425 ; *cit.* Dubreuilh, 1910.
TRAUB, E. F.—(1937) *Arch. Derm. Syph., Chicago*, **36**, 198.
TRELLES, R. A.—(1937) *Bol. Obras. San. Nacion*, **1**, 488.
ULLMANN, K.—(1898) *Wien. klin. Wschr.*, **228**.—(1900) ' Congr. Internat. Derm. Syph. Paris C.R.', t. ix, p. 221.—(1906) *Allg. wien. med. Ztg.*, **51**, 59.—(1913) *Verh. Ges. dtsch. Naturf. Arzt.*, 1913, ii, 2, p. 305.—(1914) *Wien. klin. Wschr.*, No. 12.—(1914–15) *Wien. med. Wschr.* (*cit.* Ullmann, 1917).—(1917) *Wien. klin. Wschr.*, **30**, 829.—(1922*a*) *Arch. Derm. Syph., Wien.*, **138**, 337.—(1922*b*) *Wien. klin. Wschr.*, **35**, 455, 479, 502.—(1922*c*) *Wien. med. Wschr.*, **72**, 492.—(1930) ' 8 Congr. Internat. Derm. and S., Copenhague,' p. 693.—(1932) *Wien. klin. Wschr.*, **45**, 840.—(1933) in Jadassohn, *Handb. d Haut- u. Geschl-krankh.*, Berlin, Springer, **12**, pt. 3, 628.
VICTORISZ, K.—(1931) *Biochem. Z.*, **240**, 488 ; ref. *Cancer Rev.*, 1932, 148.
v. VOLKMANN.—(1875) ' Beitr. z. Chir.,' p. 370.
VOSS, FR.—(1939) *Strahlentherapie*, **66**, 156.
WARREN, S., AND EHRENREICH.—(1944) *Cancer Res.*, **4**, 554.
WAUGH, J. F., AND SCULL.—(1935) *Arch. Derm. Syph., Chicago*, **31**, 143.

WEISKE.—(1875) *J. Landw.*
WENDE, G. W.—(1908) *J. cutan. Dis.*, **26**, 531.
WHITE, A. W. M.—(1927) *J. Cancer Res.*, **11**, 111.
WHITE, J. C.—(1885) *Amer. J. med. Sci.*, **89**, 163.—(1899) *Trans. Amer. derm. Ass.*, 1899, p. 18.
WIGLEY.—(1945) *Proc. Roy. Soc. Med.*, **38**, No. 3, Sect. Derm., 128 (discussion).
WIGNALL, T.—(1920) *Brit. med. J.*, i, 826.
WILE, U.—(1912) *J. cutan. Dis.*, **10**, 192.
WILHELM.—(1934) *Arch. Derm. Syph., Chicago*, **48**, 29, 613 (discussion).
WILHELM, L. F. X., AND GOECKERMAN, W. H.—(1943) *Ibid.*, **48**, 324.
WILSON, E.—(1871) ' Lectures on Dermatology.'
WISE, F.—(1920) *Arch. Derm. Syph., Chicago*, **2**, 197.—(1928) *Ibid.*, **18**, 809; *cit.* Anderson, 1932.—(1929) *Ibid*, **19**, 306, 697.
WOBST, A.—(1925) ' Die Huettenrauchkrankht. im Freiberger Bez. Freiberg i/S.
WRIGHT, C. S., AND FRIEDMAN, R. J.—(1933) *Arch. Derm. Syph., Chicago*, **27**, 70.
WUEHRER, J.—(1937) *Biochem. Z.*, **294**, 401.
YOUNG, E. G., AND RICE, F. A. H.—(1944) *J. Lab. clin. Med.*, **29**, 439.
YOUNG, M., AND RUSSELL, W. T.—(1926) *Spec. Rep. Ser. med. Res. Coun.*, No. 99.
ZIEL, R.—(1930) *Med. Klinik*, **25**, 623.—(1935) *J. industr. Hyg., Lond.*, **17**, 28.
ZIELER.—(1938) *Zbl. Haut- u. GeschlKr.*, **62**, 5, *cit.* Voss, 1939.
ZINNY AND VIVALDO, J.—(1942) ' Arsenicismo crónico regional endémico producido per las aguas del cono,' Buenos Ayres, p. 142.